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STUDIES ON DIPTEROUS PARASITES OF THE SPRUCE BUDWORM, CHORISTONEURA FUMIFERANA (CLEM.) (LEPIDOPTERA:TORTRICIDAE)

VI. PHOROCERA INCRASSATA SMITH (DIPTERA:TACHINIDAE)[1]

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Abstract

Phorocera incrassata Smith, which was transferred from Western to Eastern Canada for release against the spruce budworm, *Choristoneura fumiferana* (Clem.), deposits microtype eggs on leaves, which are later ingested by the host. Eggs hatch immediately after ingestion, but the parasite does not develop beyond the first instar until the host pupates. The larva then develops rapidly, matures in 10 days, and forms its puparium within the host pupal case. The adult emerges in 12 to 14 days. No information is available on the overwintering habits. Among the important characters for identifying the immature stages of *P. incrassata* are the buccopharyngeal apparatus and the anterior and posterior spiracles.

Introduction

The tachinid parasite *Phorocera incrassata* Smith was listed by Wilkes, Coppel, and Mathers (8) and by Coppel (3) respectively as fifth and seventh in importance among the dipterous parasites of *Choristoneura fumiferana* (Clem.) in British Columbia, and as eighth and 11th among all parasites of this species. It was never responsible for a parasitism of more than 1.68% (8). As it was apparently restricted to western North America, it provided an excellent opportunity for studies on the transfer of a biotic agent within a country. The species was reared at the Belleville laboratory from budworm collections in Western Canada. More than 500 adults have so far been released in Ontario, Newfoundland, and New Brunswick.

Specimens from British Columbia reared at the Belleville laboratory on *C. fumiferana* and *Pieris rapae* (L.) provided material for most of the descriptions, life history, and habits given in this paper.

Systematic Position, Distribution, and Hosts

P. incrassatus was originally described by Smith (7) in 1912, from a single female specimen collected at Moscow, Idaho, and now in the United States National Museum. Aldrich and Webber (1), in 1924, redescribed the species

and changed the trivial name from *incrassatus* to *incrassata*. Smith (7) stated that *P. incrassata* is very distinct from the other species of *Phorocera* and that the general shape of the abdomen superficially resembled that of *Zenillia blanda* (O.S.). In the Canadian National Collection the species is placed in the tribe Masicerini of the subfamily Salmaciinae (= Goniinae), where it appears to fit Mesnil's recent classification. Though this places it closer to *Eumea* (*Aplomya* of American authors), *Huebneria*, *Zenillia*, and allied genera, and not especially near the *Phorocera* complex, the character of the posterior spiracular plate of the puparium resembles that of the Sturmiini more closely than those of the *Eumea* group. Mesnil (personal communication) has commented as follows: "*incrassata* is by no means a *Phorocera* nor a Phorocerini (Exoristini), but is a Goniini (Salmaciini in Lindner); the Phorocerini produce macrotype eggs of a rather special type, whereas *incrassata* reproduces through microtype eggs; *incrassata* belongs to a genus close to *Cyzenis* R. D. from which it differs generically by its vibrissae covering more than half of the facialia [the facial ridge with strong bristles on more than the lower half] as well as by some other adult and pupal characters; *incrassata* may require the creation of a new genus". A key to the adults of the 43 species of *Phorocera* known in North America was provided by Aldrich and Webber (1) in 1924.

Before it was released in Eastern Canada, *P. incrassata* appeared to be restricted to western North America. In British Columbia collections were made by officers of the Belleville laboratory at Lillooet, McGillivray Falls, Texas Creek, Mission Mountain, and Fountain Valley.

The spruce budworm, *C. fumiferana*, is the only recorded host for *P. incrassata* in the field.

Descriptions of Stages

Adult

The adult male of *P. incrassata* is shown in Fig. 1. The detailed description of the female by Smith (7) and the redescription by Aldrich and Webber (1) are adequate.

Egg

The egg (Fig. 2) is microtype, 0.10 mm. wide, 0.18 mm. long, and brownish gray or black. It is oviform with the posterior end broadly rounded. Both dorsal and ventral surfaces are convex, the dorsal more strongly so. The ventral surface is thinner and more flexible than the dorsal. At a magnification of $40\times$ a hexagonal pattern of reticulations is visible on the dorsal and dorsolateral surfaces. The interhexagonal reticulations are deeply pigmented. The hexagonal areas are elongated and crowded together along the length of the egg in the middorsal region. A deeply pigmented ovoid area situated dorsally at the anterior end of the egg has 70 to 80 micropylar openings ($675\times$) arranged in concentric arcs (Fig. 3). The ventral surface of the egg is semitransparent and granular in appearance.
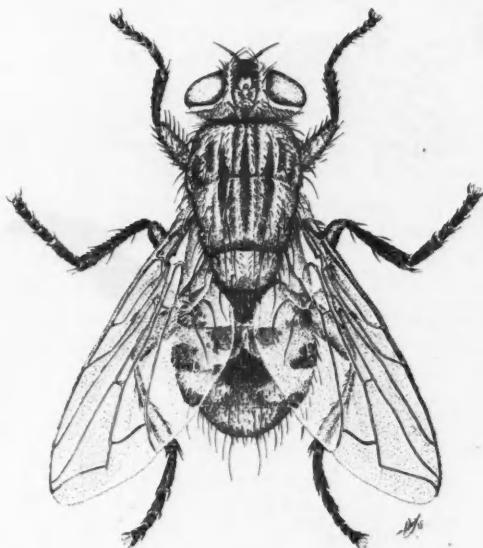
FIG. 1. *Phorocera incrassata* Smith, adult male.

First Instar

The body of the larva (Fig. 4) is similar in shape to that of *Ceromasia auricaudata* Tns. as described by Coppel and Maw (4).   It varies in length from 0.18 mm., immediately after eclosion, to 1.80 mm., when fully fed and about to molt, and in width from 0.1 mm. to 0.7 mm.   The cuticle is colorless and unpigmented.   Each segment except the terminal one bears an armature of conspicuous, black spines that are arranged in more or less continuous transverse rows (Table I). · Spines are prominent on the dorsal and lateral surfaces of the first three segments and on the ventral surfaces of all segments, except as noted above.   The longest and strongest spines are on the first three segments.   The anterior spines are directed posteriorly and the posterior ones anteriorly.

The pseudocephalon bears the antennomaxillary complex on its antero-lateral surfaces.   This complex includes a pair of two-segmented, conical antennae, two pairs of short sensory papillae, and a number of minute sensory setae surrounding the papillae.

The buccopharyngeal apparatus (Fig. 5) is well developed and is unjointed. It is strongly arched throughout the instar.   The anterior region of the apparatus is deeply pigmented.   The pigmentation extends posteriorly on both dorsal and ventral margins of the lightly pigmented central region.   The posterior region of the apparatus has lightly pigmented dorsal wings and moderately pigmented ventral wings.   The apparatus consists of two lateral wings which converge anteriorly and unite to form a single median hook (Fig. 6), which turns abruptly downward and is equipped with four to six
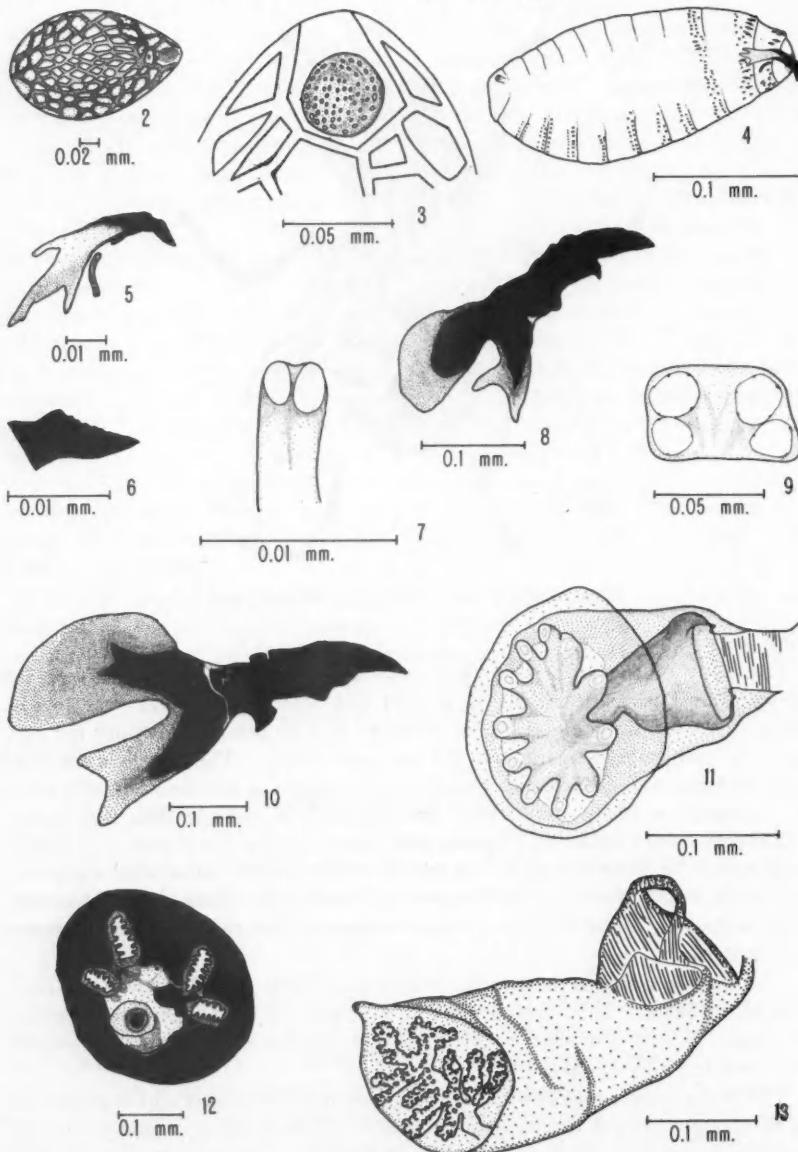
FIGS. 2–13. *Phorocera incrassata* Smith.   2. Egg, dorsal view.   3. Egg, anterior end
enlarged to show micropylar area.   4. First-instar larva, lateral view.   5. Buccopharyn-
geal apparatus of first-instar larva, lateral view.   6. Median hook, first-instar larva.
7. Felt chamber and posterior spiracle, first-instar larva.   8. Buccopharyngeal apparatus
of second-instar larva, lateral view.   9. Posterior spiracle, second-instar larva.   10.
Buccopharyngeal apparatus of third-instar larva, lateral view.   11. Anterior spiracle,
third-instar larva.   12. Posterior spiracle, third-instar larva.   13. Internal spiracle,
puparium.

TABLE I

APPROXIMATE NUMBERS OF ROWS OF SPINES ON THE DORSAL, LATERAL, AND VENTRAL REGIONS OF *P. incrassata* LARVAE

Stage	Band	Position	Segment										
			I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I	Anterior	Dorsal	2-3	2-3	2-3	0	0	0	0	0	0	0	0
		Lateral	1-2	1-2	1-2	2	0	0	0	0	0	0	0
		Ventral	3-4	2	2	2	1-2	2	1-2	2	2	1	0
	Posterior	Dorsal	0	0	0	0	0	0	0	0	0	0	0
		Lateral	0	0	0	0	0	0	0	1	1	0	0
		Ventral	0	0	0	0	0	0	0	1	1	0	0
II	Anterior	Dorsal	4-5	3-4	2-3	0	0	0	0	0	0	0	3-4
		Lateral	1-2	3-4	0	0	0	0	0	0	0	0	2-3
		Ventral	2-3	4-5	2-3	0	0	0	0	0	0	0	4-5
	Posterior	Dorsal	0	0	0	0	0	0	0	4-5	5-6	10-11	17-18
		Lateral	0	0	0	0	2-3	4-5	6-7	6-7	7-8	7-8	17-18
		Ventral	0	0	0	0	0	0	5-6	6-7	11-12	13-14	18
III	Anterior	Dorsal	5-6	5-6	1-2	0	0	0	0	0	0	4-5	0
		Lateral	2-3	3-4	1-2	1-2	1	1-2	0	0	0	4-5	2-3
		Ventral	8-9	6+3*	2-3	0	0	0	0	0	0	3-4	5-6
	Posterior	Dorsal	0	0	0	0	0	0	0	0	4-5	11-12	8-9
		Lateral	0	0	0	0	0	4-5	12-13	11-12	8-9	7-8	9-10
		Ventral	0	0	0	0	0	0	0	0	6-7	10-11	9

*Six continuous rows plus three discontinuous rows.

coarse teeth arranged in a row on its anterior edge.   Anteriorly on the dorsal margin there is a short, but moderately deep, depression, sometimes bearing one centrally placed tooth.   The dorsal wings are very short and narrow and in some specimens are difficult to discern.   The ventral wings are broad at their bases.   Each is divided posteriorly into two slightly divergent and pointed extensions.   The salivary plate is immediately below the central and posterior regions of the apparatus, and is elongate and arched to conform with the ventral margins of the apparatus.   The plate has a dorsal concavity for reception of the salivary duct.

During the latter part of this instar the central and posterior regions of the apparatus become transparent except for the elongated and lightly pigmented wing margins.

The first-instar larva is metapneustic.   There are two spiracles on the posterior surface of the terminal segment.   These are separated by a distance equal to three times their diameter.   Each spiracle (Fig. 7) has two simple openings.   The felt chambers are lightly pigmented and are visible through the body wall.   Each is approximately two and a half times as long as wide.

Second Instar

The second-instar larva resembles the first in color and form.   The length averages 2.38 mm. and ranges from 2.07 to 2.70 mm.   The cuticular armature consists of minute, semitransparent spines that are particularly numerous on the posterior margins of the five posterior segments (Table I).

The buccopharyngeal apparatus (Fig. 8) is unjointed and is deeply pigmented except for the posterior portions of the dorsal and ventral wings, which appear as semitransparent blades.   The anterior region comprises two ventrally directed, weakly curved mandibular hooks, without teeth.   The intermediate region is stout, is irregular in outline on its dorsal surface, and has two prominent, ventrally directed projections on its ventral surface. A thin, lightly pigmented bar unites the apparatus ventrally in this region. The posterior region is similar in structure to that of the first-instar larva except for the change in pigmentation of the dorsal wings.

The second-instar larva is metapneustic.   The tracheal system is extensively developed in the pleural regions and is visible through the integument. The two posterior spiracles are lightly pigmented and each has four openings (Fig. 9), which are situated in a shallow depression on the terminal segment and separated by a distance approximately two and a half times their diameters. The openings are arranged in pairs on opposite sides of the spiracular plate. Each spiracular opening unites with the common felt chamber, which is approximately three times as long as wide.

Third Instar

The third-instar larva is bluntly rounded posteriorly and tapers from the first abdominal segment anteriorly.   The length averages 5.30 mm. and ranges from 4.50 to 6.09 mm.   It is opaque and yellowish in color.   The cuticular spines are semitransparent and stouter than those of the preceding instars.   The distribution of spines is shown in Table I.

The buccopharyngeal apparatus (Fig. 10), except for a distinct articulation between the intermediate and posterior regions, is generally similar in form to that of the second-instar larva.

The third-instar larva is amphipneustic. The anterior spiracles (Fig. 11) are on the posterior margin of the prothoracic segment. Each spiracle consists of a transparent envelope enclosing the felt chamber and the terminal papillae. The atrium of the felt chamber is in the form of an inverted funnel with its apex terminating in 8 to 11 fingerlike papillae. Each papilla has a single oval orifice with an irregular inner margin. The papillae appear to be covered with a rounded, lightly pigmented cap. The posterior spiracles (Fig. 12) are in shallow depressions above the longitudinal axis of the terminal segment. Each spiracle is black and roughly circular in outline, and projects moderately above the surface of the body. The spiracles are separated by a distance of one and a half times their diameters. Each has four nearly straight slits, toothed on their inner margins and arranged in pairs on opposite sides of the spiracular plate. All slits are situated on slightly elevated and broadly rounded ridges. The fourth, or outer, slit is often shorter than the others. Three perispiracular gland openings are found between the slits. The button is prominent and is surrounded by a moderately sclerotized area.

Puparium

The puparium, which was figured by Ross (6), is approximately 4.8 mm. long and 3.2 mm. wide. It is oval and dark brown. The remains of the spine pattern of the mature larva are distinguishable on the surface of the puparium. The anterior and posterior spiracles protrude and are similar in form to those of the third-stage larva. The anal scar is oval and is situated ventrally near the posterior margin of the 10th segment. Cornicles were not located but the internal papillae possibly gain air from the general cavity of the puparium either through openings in the anterior spiracles (larval) or through thin spots in the puparial wall. Each internal spiracle (Fig. 13) has 170 to 180 respiratory papillae arranged on radiating branches at the end of the atrium.

Reproductive System

The internal reproductive system of the adult female resembles that of *C. auricaudata*, figured by Coppel and Maw (4). Each ovary contains at least 40 ovarioles, each of which is enclosed by a thin membrane and shortly after mating contains an average of 20 fully formed eggs and many smaller egg cells. The lateral oviducts are of moderately large diameter and unite to form a common oviduct that enters the anterior end of the uterus. The ducts of the three spermathecae open into the dorsal wall of the uterus at its anterior end, just anterior to those of the two accessory glands. The spermathecae are moderately pigmented. Each accessory gland consists of a kidney-shaped distal region, an enlarged central region, and a tubular duct. An extensive network of tracheoles surrounds the uterine area.

Life History and Habits

Methods

Adults of *P. incrassata* for life-history studies were obtained from British Columbia and were handled as described by Arthur and Coppel (2). They were fed crushed raisins, cube sugar, and a 10% aqueous solution of honey. Males and females were stored in separate cages and sprayed with tap water twice daily.

Observations on mating were made as with *Phryxe pecosensis* (Tns.) (5). Data on preoviposition and oviposition were obtained by placing fresh twigs of balsam or spruce in the cages. The flies oviposited freely on twigs dipped in 10% honey solution and on larvae of *Neodiprion lecontei* (Fitch) in diapause.

All rearing was conducted in the laboratory at a day temperature of 23° C., a night temperature of 15.6° C., and a relative humidity of 60%. Daily dissections of parasitized hosts provided a record of development of the parasites.

Life History

Mating was observed frequently in the laboratory. Newly emerged females mated readily with males 2 days or more old. Mated pairs remained in copula for five minutes to five and a half hours, with an average of 20 minutes for 30 pairs.

The preoviposition period varied from 10 to 34 days. The oviposition period ranged from 1 to 26 days, averaging 9 for 30 females.

The eggs are ready to hatch as soon as they are laid but must await ingestion by the host before the young larvae are freed. The eggs remained viable for at least two weeks at 23° C.

The parasite larva does not develop beyond the first instar until its host begins to pupate. Living first-instar larvae were observed in host larvae 3 weeks after eggs had been ingested. After the host pupated, the parasite spent four to six days as a second-stage larva and three to four days as a third-stage larva. Parasites reared on mature budworm larvae formed puparia approximately 10 days after ingestion as eggs.

The puparium was usually fully formed and pigmented within 24 hours. The adult emerged 12 to 14 days later, the males 1 or 2 days before the females.

The life span of ovipositing females varied from 8 to 56 days, with an average of 28 for 29 females.

Habits

Laboratory Observations

Mating, which is not necessarily accompanied by a courtship period, is similar to that of *C. auricaudata* as described by Coppel and Maw (4). To terminate mating, the female pushes the male backwards and off with her metathoracic legs.

After the preoviposition period, the female lays large numbers of eggs each day. Up to 489 eggs were laid in 1 day by a female but the average daily rate was 76. The average during the life of 30 females was 185, and the

range 1 to 1119.   Eggs are not deposited as rapidly as recorded for *C. auricaudata* (4), but 15 to 25 were laid within 30 minutes.   The female usually rests her legs on adjacent needles of spruce or balsam and oviposits on a needle in the row below.   The abdomen is lowered and the extended ovipositor is directed vertically.   Before depositing the egg the female appears to test the needle surface with her ovipositor tip.   After laying an egg the female may move away several inches only to return within one or two minutes to deposit another.   Thus two or three eggs are deposited close together before the female moves to another twig.   Females were placed with many species of lepidopterous and hymenopterous larvae, but deposited eggs freely only on larvae of *N. lecontei* in diapause and removed from their cocoons.

The method of hatching and subsequent larval development are similar to those of *C. auricaudata* (4).   The mature larva, however, does not leave the host pupal case to form its puparium.   On emergence the adults are very lightly pigmented except for the spines and bristles.   Pigmentation is complete within 12 hours.

Field Observations

The overwintering habits of *P. incrassata* are unknown, but adults appear in the field in British Columbia throughout June, when the budworm larvae are maturing.   Progeny from these adults emerge in July and early August, during and shortly after moth flight has passed its peak.   As diapause does not occur in either the host or the parasite at this time, the parasite possibly overwinters in an alternate host.   An exhaustive search in the infested area, including collecting, rearing, and dissecting larvae and pupae of possible hosts other than *C. fumiferana*, yielded no specimens of *P. incrassata*.

Superparasitism was fairly common, as indicated by the egg-laying habits. Two larval parasites and their respiratory funnels were often observed in single pupae of *C. fumiferana*, but never more than a single puparium.   No records of either multiparasitism or hyperparasitism were obtained for this species during the investigations.

Incidence of Parasitism

Most of the data on abundance of *P. incrassata* were obtained from large-scale collections of the budworm made annually in British Columbia from 1944 to 1949.   The peak year for parasitism by *P. incrassata* in the main collection areas was 1947.   Percentage parasitism was never high.   In mass collections made at Mt. McLean it was: 1944, 0.05; 1945, 0.04; 1946, 0.02; 1947, 0.50; 1948, 0.03; 1949, 0.008.   This represents up to 2.8% of the parasitism by all species.   At Texas Creek the percentage parasitism was 0.02 in 1946, 0.35 in 1947, and 0.08 in 1948, representing up to 0.54% of the parasitism by all species.   The same general situation existed at Fountain Valley from 1947 to 1949, when parasitism by *P. incrassata* was 0.07, 0.02, and 0.003% in the 3 years.   At Mission Mountain the percentage parasitism was 0.06 in 1944 and 0.08 in 1947, and was 0.16 at McGillivray Falls in 1944.

Collections of the budworm at 7-day intervals from most of the above areas showed parasitism by *P. incrassata* slightly greater than that for the mass collections. No significant differences were found in 1947–48 between various altitudes within the Douglas fir and alpine fir – Engelmann spruce zones, where the budworm population was the highest; percentage parasitism was 0.21 at 1000 feet, 0.27 at 2000, 0.36 at 3000, and 0.22 at 4000. A preliminary investigation showed that *P. incrassata* was responsible for 0.30% parasitism of budworms collected from Douglas fir and Engelmann spruce, 0.12% from alpine fir, and 0.0% from juniper.

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THE EFFECTS OF RADIATION ON THE HABITAT
TEMPERATURES OF SOME POPLAR-INHABITING INSECTS[1]

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Abstract

The relationship between habitat temperature, ambient temperature, and radiation for a series of insectan habitats on poplar leaves is shown to be highly specific. A series of these relationships is described and the possible ecological significance of the findings is discussed. The amount of heating experienced is shown to be influenced by the size and shape of the structure, and its absorptivity and ventilation.

Introduction

During recent years, as a result of the increasing interest in the field of bioclimatology, a good deal of attention has been directed toward the investigation of the microclimates within which insects must live (9). Although it is recognized that the temperature of plant parts which harbor insects is not that of ambient air, there has been little attention directed to the question of temperature characteristics of different habitats on the same plant parts. This paper is a report of such an investigation.

Wellington (8) has investigated a series of radiation effects on a variety of trees and parts thereof. Henson and Shepherd (3) have reported the effects of radiant heating of mines of the lodgepole needle miner in relation to their orientation, position, size, and color. Sullivan and Wellington (7) made comparisons of bark and leaf temperatures on poplar when the leaves were in different stages of expansion and therefore presented different exposure angles to the sun. In addition, shelters constructed by insects from silk and plant parts have had their air-space temperatures measured during different weather conditions (7, 8, 10).

A logical extension of this general approach is an investigation of the temperatures occurring in mines, leaf rolls, galls, and other types of habitats constructed or produced by different kinds of insects on one kind of leaf—in the present instance, poplar. The apparent effect of climatic change on populations of different insects all on poplar foliage might in part be the result of its effect on essentially different microclimates. The investigation constituted a part of a general investigation of bioclimatological relationships of poplar insects in the Rocky Mountains of Canada (5).

Materials and Methods

The measurements of temperature were taken by means of copper–constantan thermocouples in conjunction with a portable potentiometer with a built-in reference junction calibrated to read directly in degrees Centigrade.

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Thermocouples were made of 24- and 30-gauge duplex glass-insulated wire. Couples of suitable size and shape were made for each series of measurements. Calibration was accomplished in water against a mercury-in-glass thermometer.

Aluminum foil was used to shield leads and thermocouples used for air temperature measurement.   As a precaution against errors due to reradiation, all solid objects were removed from the vicinity of the leaves being investigated.

Two types of measurements were made.   First, spot readings of field temperatures of the various habitats were obtained under a wide variety of conditions.   Secondly, systematic measurements were made with a controlled light source in the laboratory where orientation of the leaves, air movement, and other variables could be held at a minimum.

The source of radiation used in the laboratory was a heat lamp controlled through a rheostat. This combination was calibrated against a portable meter reading in gram-calories per minute.   In the field, direct radiation measurements were taken with the same meter.

The orientation of the structures was measured with a protractor working from a horizontal surface.   Air movement was measured with a hot-wire anemometer with an omnidirectional head.   Observations made in the field at times of rapidly changing conditions were discarded in order to avoid those errors which result from the required interpolation.

Fresh leaves bearing the habitats under investigation were brought into the laboratory within minutes of the time when the observations were started. The leaves were left attached to substantial twig stubs.   Thermocouples were inserted and the series of readings was started as soon as possible.   Each series of observations was started at the lowest level of radiation, and habitat temperature was recorded at each radiation level as soon as the temperature had been the same for three 15-second intervals.   Two series of readings were taken from each leaf used and, if the second series did not duplicate the first within at least half a degree for maximum radiation, both were discarded. This was based on the assumption that a difference was evidence that the leaf had dried or changed in some other way during the first readings.

Habitats studied were, with one exception, all modifications of a single leaf or a series of leaves from a single terminal.   The study was confined to foliage of *Populus tremuloides* Michx. and, as a basis for comparison, preliminary measurements were done on single, undamaged leaves.

Results and Discussion

Undamaged Leaf

As a basis for the comparison of habitat temperatures, a preliminary study was carried out on the temperature responses of single leaves.   Thermocouples were threaded through the leaves in such a way that the junction lay in contact with the surface under study.   Leads were shielded with a wrapping of aluminum foil and run in at a low angle to the direction of radiation. The leaf surfaces were perpendicular to the radiation.

In the open, when air movement measured more than 3 m.p.h. within an inch of the leaf, the amount of heating of an undamaged leaf was rather small. At a radiation level of 1 g-cal./cm.² min., the maximum heating observed was 4 Centigrade degrees, whereas, at a level of 1.80 g-cal./cm.² min., the maximum temperature elevation was 8°. Both these values were obtained with the leaf surface at an angle of about 40° from the horizontal and at right angles to the sun's rays. Further increases of wind above 3 m.p.h. did not decrease the leaf temperature more than one degree. Decreases in wind were accompanied by increased heating but, because calm is very rare in the field, it was not possible to obtain reliable readings.

Cigarette smoke blown near the leaves being examined was carried over the surface of the leaves in a manner which suggested that rather strong convective air flow was being established over the heated surface. Such a flow would cool the leaf. In order to test the heating under conditions of minimum ventilation, single leaves were tested in the laboratory with the radiation source directed perpendicularly to horizontal leaf surfaces. Both surfaces of the leaves were exposed in this way and a complete series of readings obtained. The data are presented in Fig. 1.

The most remarkable feature of these results is the very high temperatures of the leaf surfaces. At a radiation of 1.92 g-cal./cm.² min. at the leaf surface, the temperature of the dorsal surface was 21.3° above ambient temperature and under the same conditions, the ventral surface was heated 14.2° above ambient temperature. The difference between the temperatures reached by the two surfaces is probably a function of the light color and relatively high reflectivity of the lower surface of the leaf.

Under these conditions of radiation, smoke puffs showed that, while there was a considerable amount of convection in the vicinity of the leaf, the air flow within a centimeter of the exposed leaf surface was very slight. The light winds and convective air flow over the inclined surface of the leaves which were encountered in the field would then account for a cooling of the leaf surface amounting to 13.3°.

Wellington (8) reported that the temperature of poplar leaves was 1.6° above air temperature but that these measurements were taken with the leaves hanging vertically and with considerable air movement: at least enough to maintain the leaves in "continuous movement". He suggested that if the leaves were oriented at an angle perpendicular to the sun's rays the surface temperature of the leaves probably would be greatly increased, perhaps to the same level as that reported for coniferous foliage. This suggestion was borne out first by Sullivan and Wellington's (7) data (Tables I and III) and by the present laboratory findings. The importance of angle of the leaf surface to incoming radiation also has been shown by a previous study on the temperature of mines in the needles of lodgepole pine (3). An extreme difference of 6.4° due entirely to the angle at which the needles were exposed to incoming radiation was found. The magnitude of the present effect is almost the same.
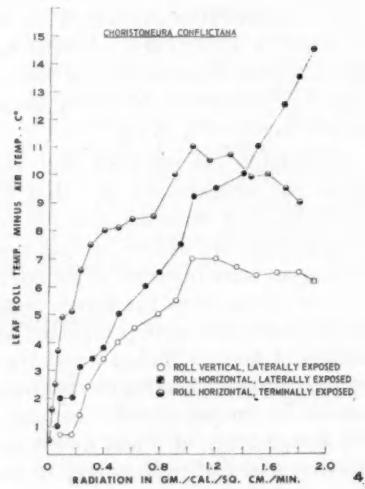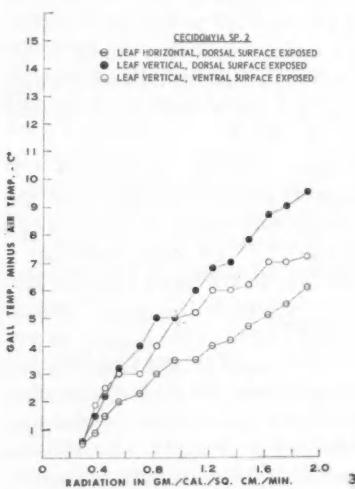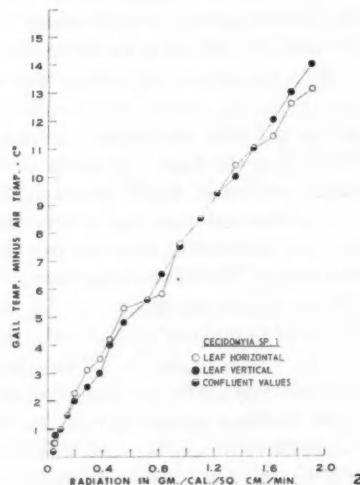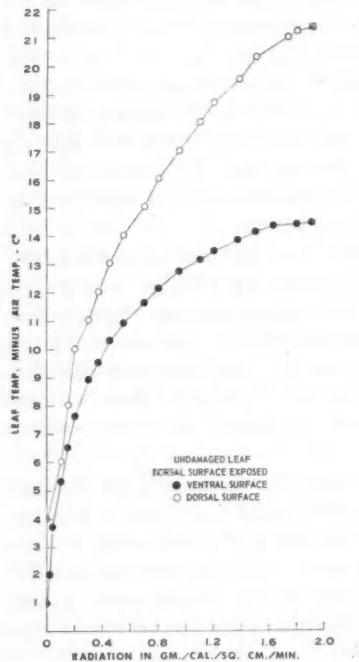
The extreme effect of relatively light air movement on surface temperatures is of particular interest. Its importance will be demonstrated when the temperature of leaf habitats which themselves stabilize the air is considered. The effect of color on surface temperature is unexpectedly large. In the study cited above (3), an extreme difference of 3.2° in the temperatures of lodgepole pine needles was found that could be attributed to color differences. In the present study, the magnitude of the difference is 7.1°.

The general form of the curves in Fig. 1 suggests that temperatures very close to the maximum which could be produced by the sun's radiation have been reached at the highest radiation value tested. The departure from linearity in the relationship is a result of increasing reradiation from the leaf and increasing convective cooling by the air. Reradiation increases as the temperature of the radiating surface increases above that of the objects to which it is radiating (4) and this factor, together with the increasing convective flow over the leaf as its surface temperature increases, combine to produce a balance. The temperature at which such a balance might be reached in a structure of different size and absorptivity could be considerably different from the balance temperature for a poplar leaf.

The time required for the leaf to reach a stable temperature increases with the increase in radiation.   A change from 0.2 to 0.8 g-cal./cm.² min. in radiation will result in a new steady temperature in the leaf within about 30 seconds.   A change from 1.2 to 1.8 g-cal./cm.² min. will result in a new steady temperature after about 90 seconds.

Cecidomyid Gall

This gall (Fig. 5), attributed to an undetermined species of the genus *Cecidomyia*, appears in the field about a month after the breaking of the poplar buds. It consists of a cavity formed by the turning of one border of the leaf. This turning is the result of a thick proliferation of mesophyll in the lower part of the leaf while the upper epidermis remains almost normal. Each gall contains one to five ivory to salmon-pink larvae which remain in the gall cavity up to the time of leaf drop.   Temperature measurements were taken within the 1×10 mm. cylindrical space of the gall cavity.   The galls were exposed with the leaf blades horizontal and with the blades vertical. The results of these measurements are presented in Fig. 2.   The direction of the radiation does not have any apparent effect on the heating of the gall.

Fig. 1.   The effect of radiation on leaf surface temperature.   Mean standard deviation of ventral surface data = ±0.5°.   Mean standard deviation of dorsal surface data = ±1.2°.   (Laboratory results.)

Fig. 2.   The effect of radiation on the internal temperature of *Cecidomyia* edge galls. Mean standard deviation = ±0.4°.   (Laboratory results.)   Normal leaf position is vertical.

Fig. 3.   The effect of radiation on the internal temperature of cecidomyid purse galls. Mean standard deviation of data = ±1.7°.   (Laboratory results.)   Normal leaf position is vertical.

Fig. 4.   The effect of radiation on the temperature of *Choristoneura conflictana* leaf rolls. Mean standard deviation of the data = ±1.2°.   (Laboratory results.)   Normal roll position is vertical.

The form of the relationship between gall temperature and radiation level is linear. Maximum radiation of 1.92 g-cal./cm.^{2} min. resulted in a net heat gain of 14° above ambient temperature.

The difference between the amount of heating and the form of the curve in this gall and the same relationships in the undamaged leaf indicate that cooling of the leaf by air movement (convective) is the most important influence in the moderation of undamaged leaf temperature. In the cecidomyid gall, convective cooling is apparently relatively slight because of the volume of tissue and enclosed air in the gall. The absorptivity of the gall is similar to that of the ventral surface of the undamaged leaf.

Cecidomyid Purse Gall

This gall, caused by a second undetermined species of the genus *Cecidomyia*, consists of a purse-shaped proliferation of leaf tissue with a long slit opening to the upper surface of the leaf (Fig. 6). For the most part, the galls are blood-red on the underside and scarlet on the upper surface. Each commonly contains only one larva. Temperature measurements were taken within the tear-drop-shaped cavity of the gall with the leaves bearing the galls exposed to radiation from the bottom and the top. The results of these measurements are presented in Fig. 3.

The amount of heating produced by dorsal exposure of the gall with the leaf horizontal is minimal. Dorsal and ventral exposures with the leaf vertical resulted in more heating, with dorsal exposure slightly more effective than ventral. The magnitude of the difference between dorsal and ventral exposure is proportionately similar to that in the undamaged leaf and probably has a similar origin.

The amount of heating of this gall is small in comparison with the heating of an undamaged leaf. The subspherical shape of the structure and consequent relatively low surface–volume ratio is probably the best explanation of this slight heating effect. A size effect can also be demonstrated in the case of a structure to be discussed below which is subspherical and is found in a variety of sizes.

Choristoneura conflictana Leaf Roll

The leaf roll produced by larvae of *Choristoneura conflictana* (Wlk.) is illustrated in Fig. 7. Rearing of larvae from such rolls yielded two larvae of *Sciaphila duplex* Wlshm. from a total of 60 rolls. There is a possibility that some of the rolls used in the experiments and attributed to *Choristoneura* were caused by the less common insect. The roll is tied by silk, but is open at both ends. Its axis may be either at right angles to or parallel to the axis of the leaf. Rolls frequently contain considerable quantities of frass. The larva lives in a silk-lined cylindrical chamber in the center of the roll and the temperature measurements were made there.

The results of the measurements are presented in Fig. 4. When the roll was horizontal and receiving radiation from the side, the relationship between radiation level and the temperature of the roll was roughly linear. The
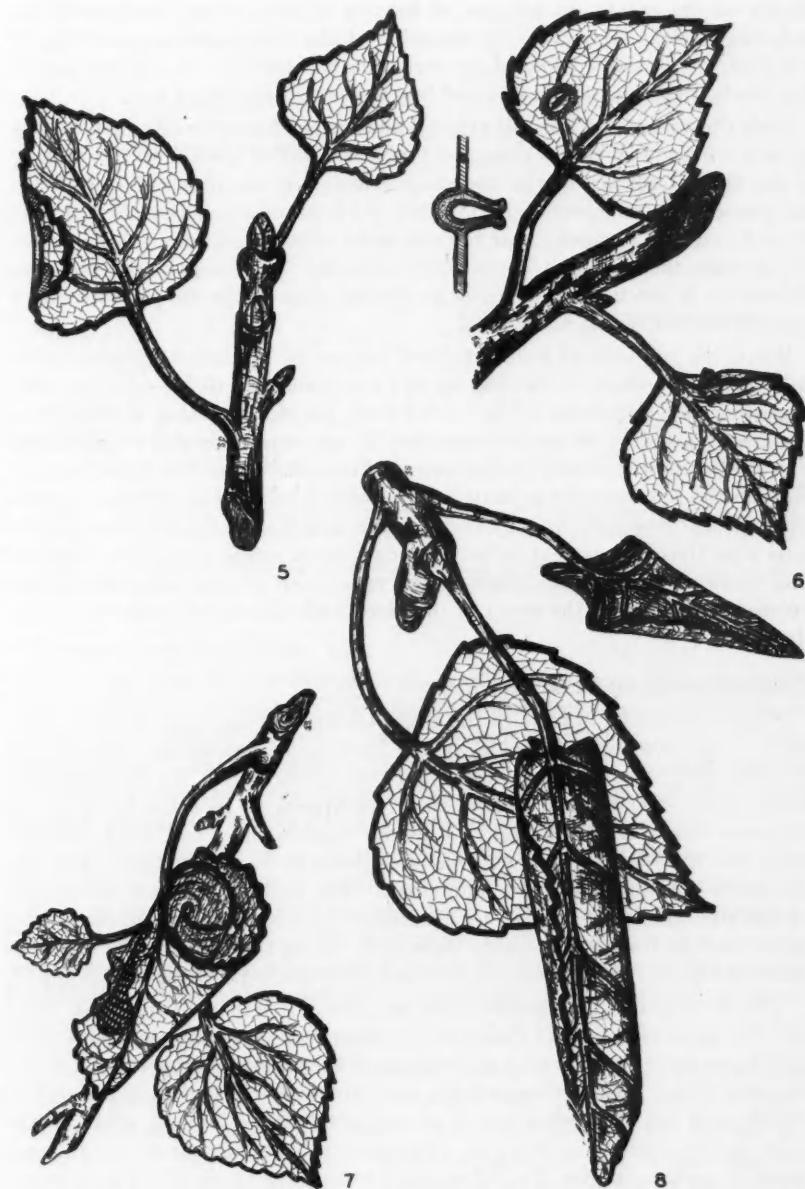
Fig. 5. Edge gall; *Cecidomyia* sp.   Fig. 6. Purse gall of *Cecidomyia* sp.   Fig. 7. Leaf roll of *Choristoneura conflictana*.   Fig. 8. Leaf roll of *Compsolechia niveopulvella*.

amount of heating with maximum radiation under these conditions was similar to the maximum amount of heating of the ventral surface of the undamaged leaf.   However, the linearity of the relationship shows that in this case, convective cooling of the structure was ineffective.   At low radiation levels, the structure was heated less than the undamaged leaf.

With the axis of the leaf roll vertical and heated from the side, the heating up to a radiation level of 1 g-cal./cm.² min. was rather similar to the heating of the ventral surface of the undamaged leaf.   Above that radiation level, the temperature of the roll falls.   This is evidence of a chimney effect within the roll.   A puff of smoke near the roll under these conditions will be drawn up through the roll at a considerable velocity.   Apparently the chimney circulation is much more effective in cooling than is the simple convection over the surface of the normal leaf.

When the roll is held horizontal and heated by radiation directed at its open end, the amount of heating up to a radiation level of 1 g-cal./cm.² min. is greater than when the roll is heated from the side.   Above this level, an increase in radiation is not accompanied by an increase in roll temperature. The behavior of a smoke puff shows that convective air flow is established through the roll from the unheated to the heated end.   The rolls were heated from the leaf base end, which was commonly larger than the distal end.   The rolls were thus of a conical rather than cylindrical shape and convective flow was made possible by the slope of the upper part of the roll.   The initial excess of heating was the result of the direct radiation of the inner surface of the roll.

Compsolechia niveopulvella Leaf Roll

A leaf roll similar in form to the *Choristoneura* roll but different in two important characteristics is made by the larvae of *Compsolechia niveopulvella* Chamb. (Fig. 8).   In some areas, such as Leanchoil in the Kickinghorse Pass, almost half the leaf rolls of this type appear to be made by larvae of *Epinotia nisella criddleana* Kft.   Rolls for experimental uses were collected from the Bow Valley where *Epinotia* appears to be uncommon.   The leaf is tightly tied and the roll compressed.   The ends of the roll are tightly closed with silk.   Larvae live within the roll and temperature measurements were made in the central cavity without breaking the walls of the roll more than necessary.   The results of these measurements are presented in Fig. 9.

The heating of *Compsolechia* rolls is a linear function of radiation.   The amount of heating at full radiation is rather low in comparison with other structures tested and there is no evidence of the chimney effect seen in *Choristoneura* rolls.   In the *Compsolechia* rolls, the ends of the leaves are tied so tightly and the whole structure is so compact that no chimney effect is possible in any position of the roll.   The general compactness of the roll is a possible explanation for the rather small amount of heating.   When the roll is heated from the end, the amount of internal temperature increase is very small and irregular.
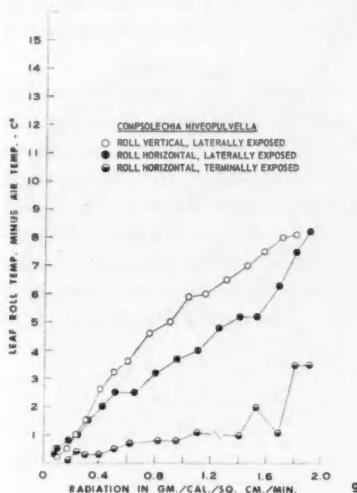
FIG. 9. The effect of radiation on the temperature of *Compsolechia niveopulvella* leaf rolls. Mean standard deviation of data = ±1.7. (Laboratory results.) Normal roll position is vertical.

FIG. 10. Effect of radiation on the temperature of *Phyllocnistis populiella* mines. Mean standard deviation of data = ±1.1°. (Laboratory records.)
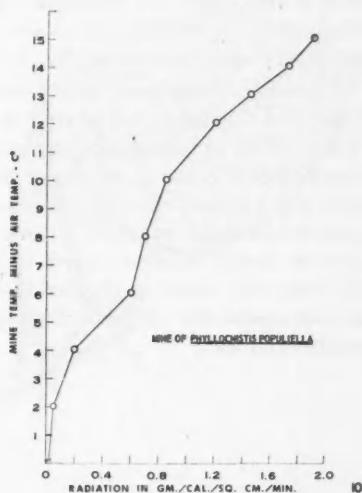
Phyllocnistis populiella Mine

The mine made by *Phyllocnistis populiella* Chamb. is an extensive modification of the leaf structure. The mesophyll and lower epidermis are mined out and the palisade layer and upper epidermis together with the lower cuticle remain. The structure is illustrated in Fig. 13. A small layer of air is enclosed by the lower cuticle and it was within this very small space that the temperature measurements presented in Fig. 10 were made.

The relationship between temperature of the mine and radiation is close to linearity. The amount of heating at maximum radiation is similar to the amount of heating found with horizontal orientation and lateral exposure of the *Choristoneura* leaf roll. The implication of this finding is that the convective cooling which so effectively modifies the temperature of leaf surfaces is ineffective in the modification of the temperature of any enclosed air space.

Choristoneura conflictana Tied Leaves

Newly emerged larvae of *Choristoneura conflictana* feed in a gallery formed from two leaves which are tied together with silk (5). For the most part the feeding is confined to one of the leaves and the cuticle is left so that the feeding space is walled on one side by undamaged leaf and on the other by cuticle (Fig. 14). Frequently, as illustrated, there is an island of undamaged epidermis on the mined side of the leaf. This tissue is supported by the surrounding cuticle. The structure as a whole is similar to the *Phyllocnistis*

mine in that there is a relatively thick layer of leaf and a very thin layer of cuticle enclosing a thin layer of air.   Temperature measurements made within the gallery are presented in Fig. 11.

The relationship between temperature of the gallery and radiation level is linear and the amount of heating is extreme—in the same order of magnitude as that of the undamaged leaf surface when convection was held to a minimum. However, the form of the curve is very similar to that of the *Phyllocnistis* mine and I believe that the difference lies only in the size of the air space and mass of leaf tissue left undisturbed.   The effect of radiation on mine temperature is similar without regard to the side of the structure which is heated. The very thin covering afforded by the cuticle is probably almost transparent so that essentially all the radiation from either direction is absorbed by the undamaged leaf.
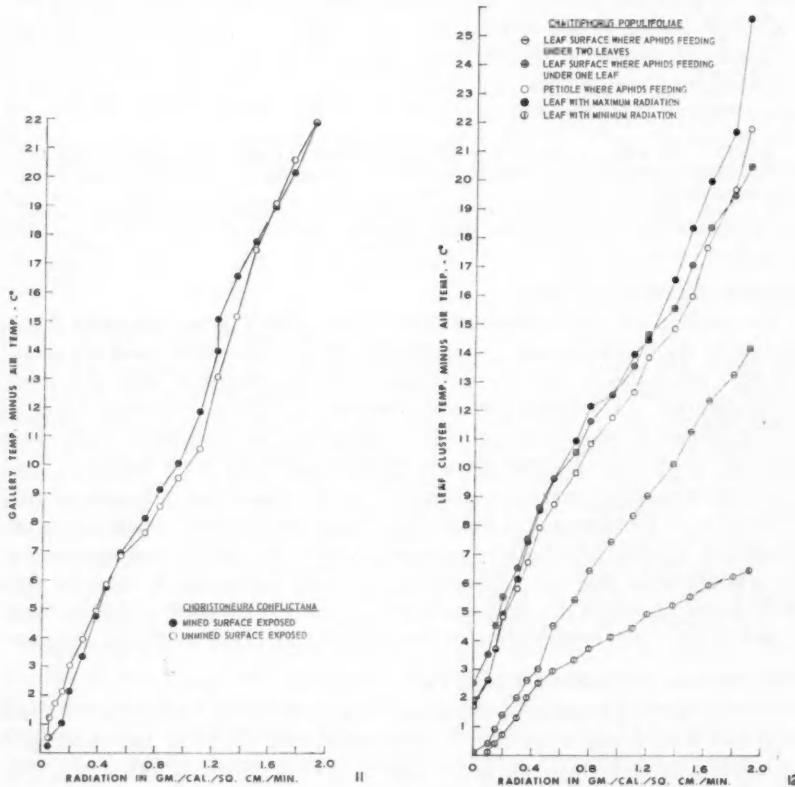
FIG. 11.   The effect of radiation on the temperature of *Choristoneura conflictana* leaf gallery.   Mean standard deviation of data = ±0.7°.   (Laboratory results.)

FIG. 12.   Effects of radiation on the temperature of terminal modified by *Chaitophorus populifolia*.   Mean standard deviation of data = ±1.3°.   (Laboratory results.)
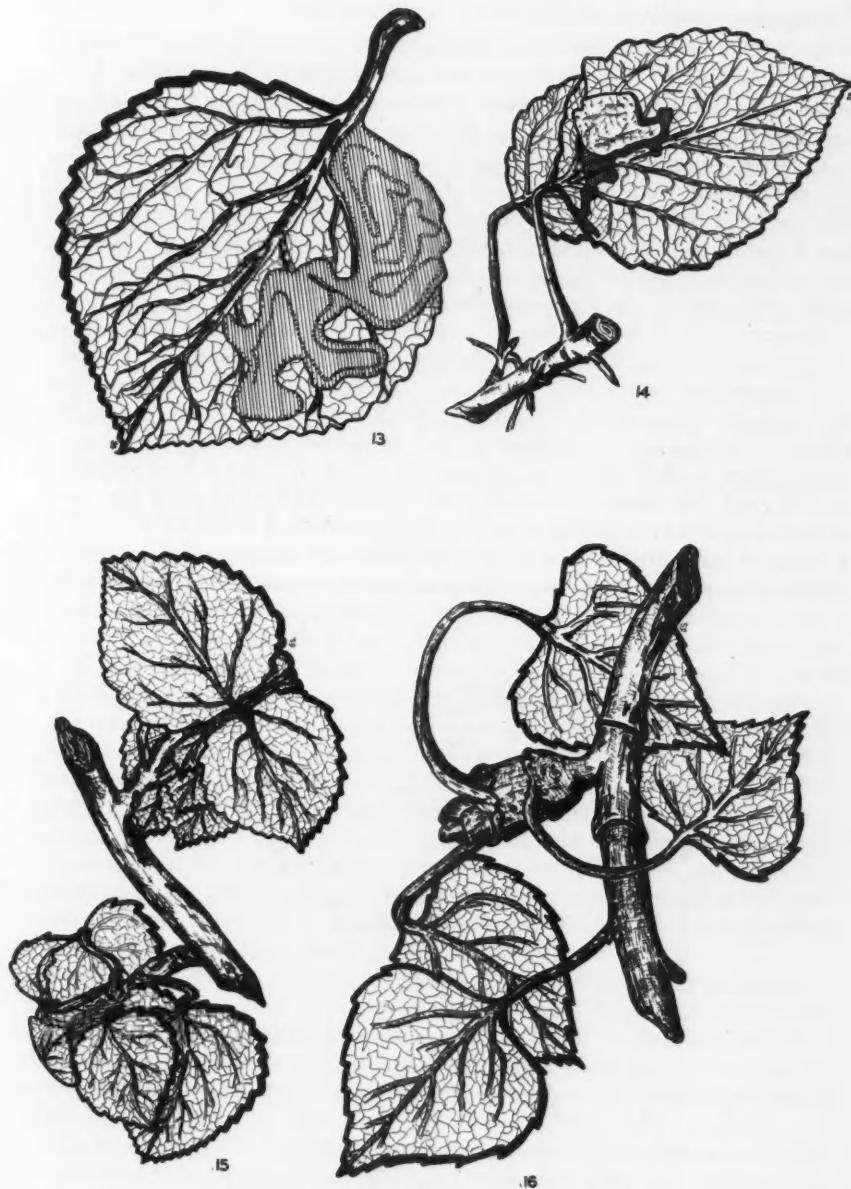
FIG. 13.   Mine of *Phyllocnistis populiella*.
FIG. 14.   Feeding gallery of *Choristoneura conflictana* larvae.
FIG. 15.   Poplar terminals affected by *Chaitophorus populifolia* Davis.
FIG. 16.   *Agromyza schineri* gall.

Chaitophorus populifolia Colony

The colony of *Chaitophorus populifolia* Davis involves the entire terminal where it is found. The feeding of the aphids distorts the petioles of the leaves in such a way that the leaves of the terminal are closely appressed and held together in a compressed and flattened bunch (Fig. 15). Within such leaf clusters, temperatures were taken in a variety of places while radiation was consistently from the side, with the leaves arranged in a vertical rank. The bodies of the aphids occupy a considerable amount of space between the leaves and the wax filaments they produce occupy additional space.   Because this affects movement of air through the spaces between the leaves, the aphids were left in position during temperature measurements.

Measurements of temperature in a variety of positions within the colonies are presented in Fig. 12.   The form of the relationships between radiation and temperature at a variety of locations in the aphid colony was linear but the ultimate temperatures varied considerably among locations within the colony.   Heating of the exposed surface of the outer leaf was extreme; a temperature of 25.5° above the ambient temperature resulted from radiation at 1.92 g-cal./cm.² min.   This value, which is even higher than that measured on undamaged leaves, probably reflects both the effect of stabilized air and the backing of the heated surface by layers of relatively opaque leaves.   Surfaces within the cluster decrease in temperature with increasing distance from the
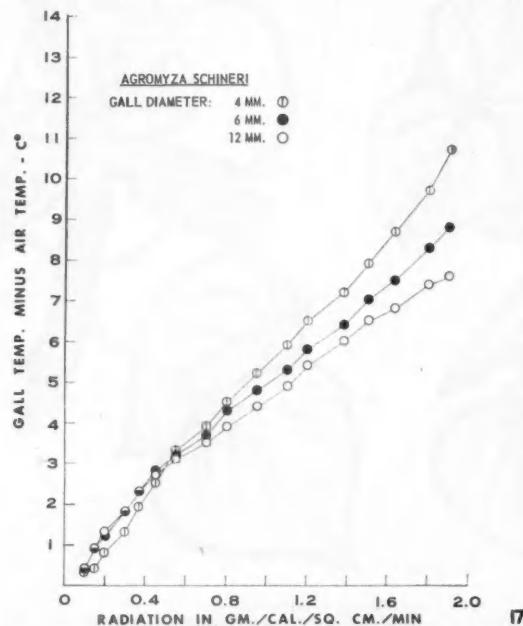

FIG. 17.   Effect of radiation on the temperature of galls of *Agromyza schineri* Gir. Mean standard deviation of data = ±0.4°.   (Laboratory results.)

illuminated surface. This results in rather a sharp gradient of almost 19°
between the illuminated surface and the shaded outer surface at an illumination
of 1.92 g-cal./cm.² min.

Agromyza schineri Gir. Gall

The subspherical swellings in the terminal twigs whjch constitute this gall
are illustrated in Fig. 16. The relationship between temperature of the interior
of the gall, size of gall, and radiation intensity is illustrated in Fig. 17. These
temperature measurements were taken as close as possible to the center of
the galls. The color of the galls was very constant (that of normal twig bark)
and the shape of all galls examined was very similar. Measurements of gall
diameters were taken at right angles to the axis of the twig and at the equator
of the gall.

The relationship between gall temperature and radiation appears to be linear
in all cases. The amount of heating is moderate in comparison to the extent
to which some leaf structures are heated. The smallest galls were heated
most and the largest, least. This is probably an effect of the different surface–
volume ratios in the different sizes; the highest surface–volume ratio results
in the greatest heating.

Discussion

Aside from orientation, a number of factors seem to determine the amount
of heating which is accomplished by a standard amount of radiation falling
on the insectan habitats discussed. The shape of the structure, air circulation
at its surface or within, surface–volume ratio, color or absorptivity, and
possibly other characteristics all have an effect on the habitat's temperature.

The air cooling and radiational cooling of an undamaged leaf combine to
produce a balance between radiant heating and the various sorts of cooling
beyond which further increases in radiation probably cannot increase the tem-
perature of the leaf (at least within the limits of radiation values encountered
in nature). In any insectan habitat examined, the modification of the leaf
had so altered the cooling behavior that the sort of balance shown by the
undamaged leaf did not appear. However, in almost every habitat, the tem-
perature at the place where the insect lived never reached the extreme values
found on the exposed surface of an undamaged leaf.

Maximum radiant heating is found in structures where air is stabilized in
thin layers between thin sheets of tissue and where the air spaces are com-
paratively small. Minimal radiant heating is found in structures where a
large volume of air is partially enclosed by tightly packed or dense tissue. In
solid structures, internal temperature increase due to radiant heating decreases
with increasing size.

Where enclosed air is susceptible to rapid turnover from chimney or other
special convective processes, the cooling effect of the circulating air is maximal.

Orientation of the exposed surface of the leaves in such a way that ventilation
is increased by convective circulation of the air has a profound influence on
cooling of irradiated structures. Wind up to 3 m.p.h. produces profound
cooling but further increases in wind have little effect.

In the area where these studies were conducted, air temperatures are most often rather low, even during the peak of the growing season. Average maximum temperatures for July (the hottest month) are near 25° C. (77° F.), while the average maxima for other months of the growing season are very much lower. Moreover, the amount of variation in temperature from season to season is extreme. In such an area, the ecological importance of the habitat temperature characteristics which have been described may be very great. Radiant heating of the habitat often is the factor which permits development of the insects during a considerable part of the season. This fact is demonstrated by the observation that considerable amounts of activity are common before the air temperature as recorded by shielded thermographs has risen to the physiological minimum. It is possible, though not yet demonstrated, that during dry, sunny seasons, excessive heating of the habitats of immobile insects may cause mortality. Certainly, excessive heating of the habitats of the more mobile insects causes extensive movements of the animals. For example, when the habitat temperature of *Choristoneura conflictana* rises to 36° or higher, the insects fall from their leaf rolls. Certainly this must result in the death by starvation of some of the affected insects.

The cecidomyid edge gall was subject to moderate heating while the cecidomyid purse gall was subject to only slight heating. The most obvious inference is that the first species would often have an advantage over the second so far as speed of development is concerned and that this advantage would be greatest when the air temperature was at or below the physiological zero. It will be of considerable interest to test this and other inferences by detailed observation of population changes associated with annual climatic changes.

Perhaps the most interesting comparison to be made between the various habitats studied is that between the leaf rolls made by *Compsolechia* and by *Choristoneura*. As we have seen, the only obvious difference between these structures lies in the tighter rolling and the end closure of the *Compsolechia* rolls. The heating of vertically suspended rolls of both types is similar up to a radiation value of 1 g-cal./cm.² min., with the *Choristoneura* roll being heated slightly more than that of *Compsolechia*. Beyond this point, however, the responses of the two rolls are very different, with the *Choristoneura* roll being cooled by extreme convective flow which results from a chimney effect. At the same time, the closed *Compsolechia* rolls continue to be heated to a final temperature in excess of that reached by *Choristoneura* rolls.

Choristoneura rolls suspended horizontally and heated from the side were heated about twice as much as *Compsolechia* rolls treated in the same way. *Choristoneura* rolls suspended horizontally and heated from the end showed a temperature response similar to that of the same structure suspended vertically, i.e., some cooling by chimney effect. However, *Compsolechia* rolls suspended horizontally and heated from the end were only very slightly affected.

From this we see that the temperature regime in *Choristoneura* rolls will depend on the mean orientation of the individual roll to the sun's rays. Most of those observed in the field have been hanging vertically. In this position, there is strong heating at lower levels of radiation and a relatively stable

temperature at high levels of radiation. By contrast, *Compsolechia* rolls, which also tend to be vertically suspended, are heated less than the *Choristoneura* rolls in this position at low levels of radiation, but more at high levels. Any change in microclimate or other behavioral determinant at the time of roll construction in either species might have an effect on the form of the roll.

It has been noted already that the roll axis may be either at right angles to or parallel to the axis of the leaf. It may be seen on the basis of the present findings that such differences in the rolls, though they may appear trivial, have profound effects on the roll microclimates and perhaps on the survival of the species involved.

The tied leaves of *Choristoneura conflictana* first-instar larvae provided the hottest enclosed environment examined. In view of the fact that these tied leaves are inhabited during the hottest part of the year, it seems probable that the amount of heating experienced at times may force the insects from their habitats. During sampling, large numbers of empty mines were found at a time when completion of this phase of growth was improbable. This may be evidence that some mines at least are deserted during severe heating.

The heating of the *Phyllocnistis* mine is a simple process and requires no comment. The ecological significance of the heating would be simply that activity of the insect is possible under conditions of even moderate insolation at times when the ambient temperature was considerably below the physiological zero. Thus, a sunny season would be of some advantage to this insect. A similar significance may be assumed for the heating of the *Agromyza* galls, though the amount of heating of these structures is relatively low and the advantage of sunny weather would be correspondingly slight.

The heating of the *Chaitophorus* habitat is extreme. Interestingly enough, a very large gradient exists between the heated and the shaded sides of the habitat and the inhabitants would thus be presented with a considerable range of temperature choices. Sunny weather would be of maximum significance to these aphids; it is improbable that they could be overheated under the usual growing season conditions found in the Canadian Rocky Mountains. If the heated side of the cluster became overheated, the insects could move to a cooler part of their leaf cluster. On the other hand, under sunny conditions, even if the ambient temperature was very low, a relatively favorable temperature regime could be found somewhere in the leaf cluster.

Conclusions

1. The temperature of different kinds of insectan habitats on the same host and constructed on the same host part cannot be assumed to be the same.

2. Aspect, exposure, orientation, ventilation, absorptivity, density, and size all affect the temperature of an insectan habitat under the influence of radiation and cause it to be different from the temperature of undamaged host tissue.

3. The character and consequent temperature response to radiation of the insectan habitats examined is species specific and the ecological significance of this characteristic should certainly be considered in any description of a species.

Acknowledgments

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LA TOXICITÉ DU DDT POUR LE SAUMON DE L'ATLANTIQUE (SALMO SALAR LINNÉ) ET LES ALEVINS DE TRUITE (SALVELINUS FONTINALIS MITCHILL)[1]

ANDRÉ GAGNON[2]

Abstract

The relative sensitivity of brook trout fry, salmon fry, and 1- and 2-year-old parrs to known quantities of DDT was studied in hatchery-trough experiments with larger numbers of specimens than were used by previous workers. In troughs, in which part-time water circulation was achieved and DDT used at the rate of 0.5 lb./acre (0.32 p.p.m.), high mortality rates (93–100%) were recorded for salmon fry, trout fry, and 1-year-old parrs. Moreover, it was shown that group susceptibility decreases in the following manner: salmon fry > 1-year-old parrs > trout fry. In another series of experiments, under identical conditions, it was shown that sensitivity in salmon decreases with age. Using salmon fry, in troughs deprived of water circulation, a study was made of the effects of various concentrations of DDT: 0.33, 0.18, 0.072, and 0.036 p.p.m. corresponding to 0.5, 0.25, 0.1, and 0.05 lb. of DDT/acre, respectively. The average lethal dose (L.D. 50) was found to be 0.072 p.p.m. A comparison between two series of experiments shows how mixing of the DDT with water makes the poison more deadly than when used as a DDT–oil solution on the surface of water.

Introduction

Depuis une douzaine d'années de nombreuses observations faites en milieu naturel et des expériences de laboratoire ont démontré la toxicité du DDT pour certaines espèces de poissons (2, 4, 5). À ces nombreuses données, il nous a semblé utile d'en ajouter quelques-unes se rapportant au saumon de l'Atlantique, *Salmo salar*, et à l'alevin de truite, *Salvelinus fontinalis*.

Nous avons fait ce travail au mois d'août 1955. À ce moment-là, on trouvait déjà dans la documentation scientifique quelques résultats relatifs à l'alevin (6) et au fretin (8) de *S. fontinalis*. Plus récemment, Hatch (2) a publié des données se rapportant aux alevins de *S. salar*, *Salmo gairdneri* et *S. fontinalis*. Cependant, tous les résultats d'expériences de laboratoire, publiés à date, ont été obtenus avec de très petits nombres d'individus; aussi croyons-nous que nos expériences, réalisées avec des nombres plus considérables de sujets, présentent un intérêt assez particulier.

Par ce travail, nous nous sommes proposés de déterminer laquelle des deux espèces d'alevins, de saumon ou de truite, est plus sensible au DDT. Nous avons aussi étudié la sensibilité relative de jeunes saumons d'âges différents, à une même concentration de DDT. Enfin, nous avons cherché à établir une corrélation entre le taux de mortalité chez des alevins de saumon et la teneur en DDT du milieu dans lequel on les place.

Matériel et Méthodes

Les alevins de saumon, d'une longueur moyenne de 41.87 ± 0.28 mm., et les alevins de truite, d'une longueur moyenne de 61.20 ± 0.99 mm., avaient 3 mois.   Nous les avons obtenus de la Pisciculture provinciale de Gaspé, située à Sunny Bank, où nous avons fait tout le travail expérimental.   Tous ces alevins provenaient des mêmes groupes en élevage.

Les saumoneaux, au stade parr, furent capturés à la senne sur la rivière York, à proximité de la pisciculture.   Ils furent gardés dans les bassins d'élevage au moins une semaine avant les expériences.   D'ailleurs, il convient de mentionner que nous n'avons perdu aucun parr en captivité, laquelle a duré 5 semaines pour certains individus.   En tout temps, ils nous ont paru en excellent état.   Puisque dans le cas des parrs, nous voulions effectuer des expériences chez deux groupes d'individus d'âges différents, nous avons procédé de la façon suivante.   Selon le cas, nous avons choisi parmi les parrs en captivité soit de petits individus, ayant à peu près une même taille, ou encore, des individus nettement plus gros et aussi de taille plus ou moins semblable.   Après les expériences, chacun d'eux fut mesuré et conservé pour être soumis à un spécialiste qui en détermina l'âge.*   Ceux du premier groupe, d'une longueur moyenne de 70.98 ± 0.56 mm., avaient 1 an, tandis que ceux du deuxième groupe, d'une longueur moyenne de 117.61 ± 0.93 mm., avaient 2 ans.

L'eau utilisée au cours des expériences était de même provenance que celle des bassins d'élevage de la station piscicole et nous en avons suivi les variations de température.   Nous avons aussi fait des mesures de pH, à l'aide d'un appareil Photovolt, et des dosages de l'oxygène dissous selon la méthode de Nicloux (7).   Toutes ces mesures portaient aussi sur l'eau des bassins témoins, l'un de ceux-ci servant à l'élevage en cours.

Les expériences ont été effectuées dans des bassins ordinaires d'élevage, de forme rectangulaire, mesurant 11 pieds de longueur, 1 pied et 8 pouces de largeur et 9 pouces de profondeur.   Nous avons eu soin de calculer, pour chacun des bassins, la surface et le volume de l'eau afin d'utiliser des quantités déterminées de DDT.   Les bassins contenaient en moyenne 10 pieds cubes d'eau.   La même eau servait pour toute la durée d'une expérience.   Nous avons constaté que cela ne présentait aucun inconvénient.   Dans toutes nos séries d'expériences, sauf une sur laquelle nous reviendrons plus tard, nous avons utilisé quatre bassins aménagés avec deux systèmes de pompage.   Trois d'entre eux, contenant l'eau et la solution de DDT, se partageaient un même système, tandis que le quatrième bassin possédait un système de pompage séparé puisqu'il devait servir de bassin témoin.   De l'une des extrémités de chacun des bassins le liquide passait dans une pompe qui le poussait à l'autre extrémité où il tombait en giclant.   Ces systèmes fonctionnaient à intervalles assez réguliers; ils assuraient une bonne circulation dans les bassins, enrichissaient l'eau en oxygène et mélangeaient la solution de DDT à l'eau.   Ces conditions, nous semble-t-il, rappellent un peu ce qui se passe en eau rapide

*Ces déterminations furent faites par monsieur Léon Tremblay, de la Station de Biologie Marine, à Grande-Rivière, Québec.

lors des vaporisations.   Ajoutons que le débit d'eau était essentiellement le même dans chacun des bassins ainsi alimentés.   Ces quatre bassins étaient recouverts d'un treillis métallique.

Quelques heures avant l'addition de la solution de DDT, les poissons étaient placés dans leurs bassins respectifs.   Ainsi, on aurait pu éliminer, avant l'addition du DDT, les sujets en mauvais état résultant de la manipulation pourtant soignée.   Cette difficulté ne s'est jamais présentée.   Enfin, signalons que les poissons ne recevaient aucune nourriture durant les expériences.   En agissant ainsi, nous avons voulu éviter toute perte de DDT soit directe, par élimination pendant le nettoyage des bassins, soit indirecte, par adsorption par les particules alimentaires.

Le seul critère de toxicité que nous ayons employé fut la mort des sujets. Les dénombrements des morts furent fréquents, comme l'indiquent les figures ci-après.   Mentionnons toutefois que les symptômes d'intoxication au DDT (6) pouvaient être observés chez les individus affectés, chez qui la mort suivait habituellement de très près.   En dénombrant les morts nous avons donc noté le nombre d'individus présentant ces symptômes, afin de prévoir le taux de mortalité pour de courtes périodes à venir.

La solution de DDT et le solvant pur, soit l'huile Picco Hi-Solv no 473, nous ont été gracieusement fournis par monsieur B.W. Flieger, qui avait charge de l'exécution des vaporisations aériennes au DDT, en Gaspésie.   Un gallon américain de solution contient une livre de DDT technique.   Pour obtenir une quantité de DDT équivalente à 0.5 livre/acre, il nous fallait prendre 0.8 cc. de solution.   L'addition de la solution ou du solvant pur, selon le cas, se faisait à l'aide d'une pipette de façon à ce que les gouttes se répartissent uniformément sur toute la surface aqueuse.   Pour des concentrations inférieures à 0.5 livre/acre, nous avons fait les dilutions telles qu'on puisse additionner le même volume de solution.

Résultats

Considérons d'abord les résultats que nous ont donnés les expériences effectuées dans les bassins avec systèmes de pompage.   Rappelons que le DDT y fut employé à raison de 0.5 livre/acre, soit 0.32 partie par million (p.p.m.),* dans les conditions où nous avons opéré.   Les résultats de deux des trois séries d'expériences sont exprimés dans les courbes des figures 1 et 2.   Celles-ci démontrent que les alevins de saumon et de truite, les parrs de 1 an et de 2 ans sont tous plus ou moins affectés par le DDT.   Chez les alevins et les parrs de 1 an, le taux de mortalité est très élevé, soit de 85 à 100%.   Chez les parrs de 2 ans, le taux de mortalité est très bas, soit 8% (fig. 2), pour une période expérimentale de même durée.   Cependant, même lorsque la mortalité est très élevée, nous voyons que la sensibilité varie d'un groupe à l'autre.   Ainsi, les alevins de saumon sont les plus sensibles, et les parrs de 1 an sont plus sensibles que les alevins de truite (fig. 1).   Ces résultats ont été confirmés par une autre série d'expériences.

*p.p.m. représente le poids de DDT, exprimé en milligrammes, par litre d'eau.
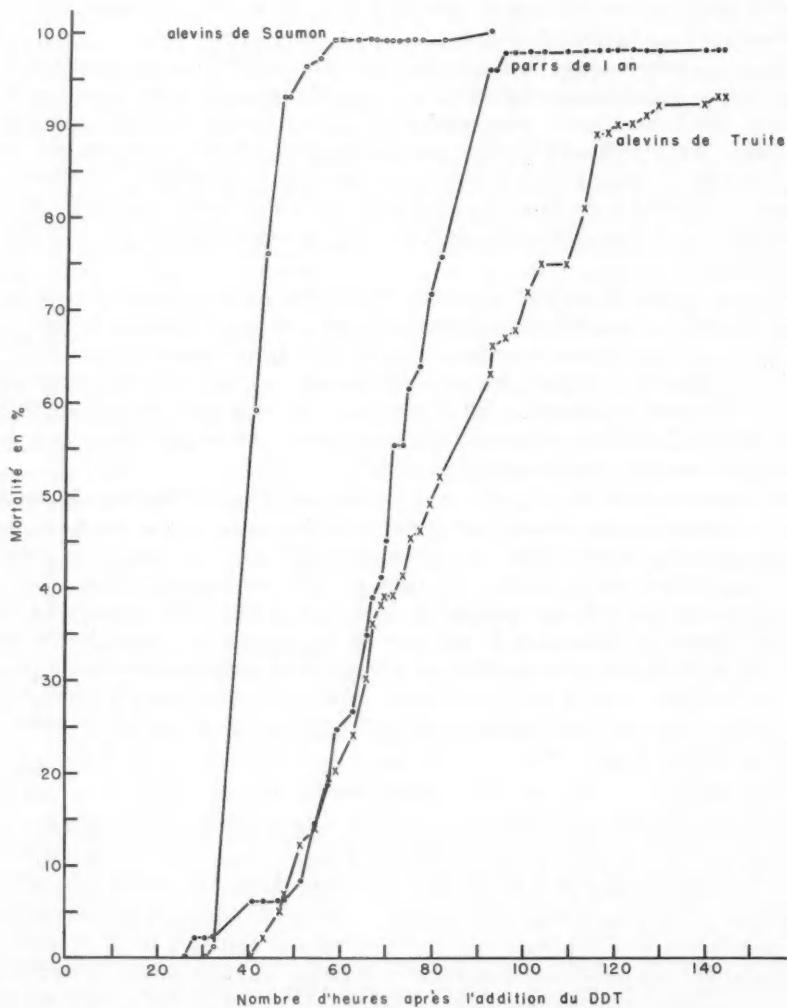
FIG. 1.   Courbes de mortalité chez 100 alevins de saumon, 100 alevins de truite et 49 parrs de 1 an.
   Concentration en DDT: 0.5 livre/acre (0.32 p.p.m.).
   Températures: 13.0°–22.0° C.
   Témoins: un taux de mortalité de 1% enregistré dans une expérience effectuée simultanément chez 99 alevins de saumon, en eau pure, dans le bassin avec système de pompage séparé.   Aucune mortalité enregistrée au cours d'expériences témoins effectuées dans les mêmes bassins, avec les mêmes nombres d'individus, alors que le Picco Hi-Solv seul fut substitué à la solution de DDT-Picco Hi-Solv.
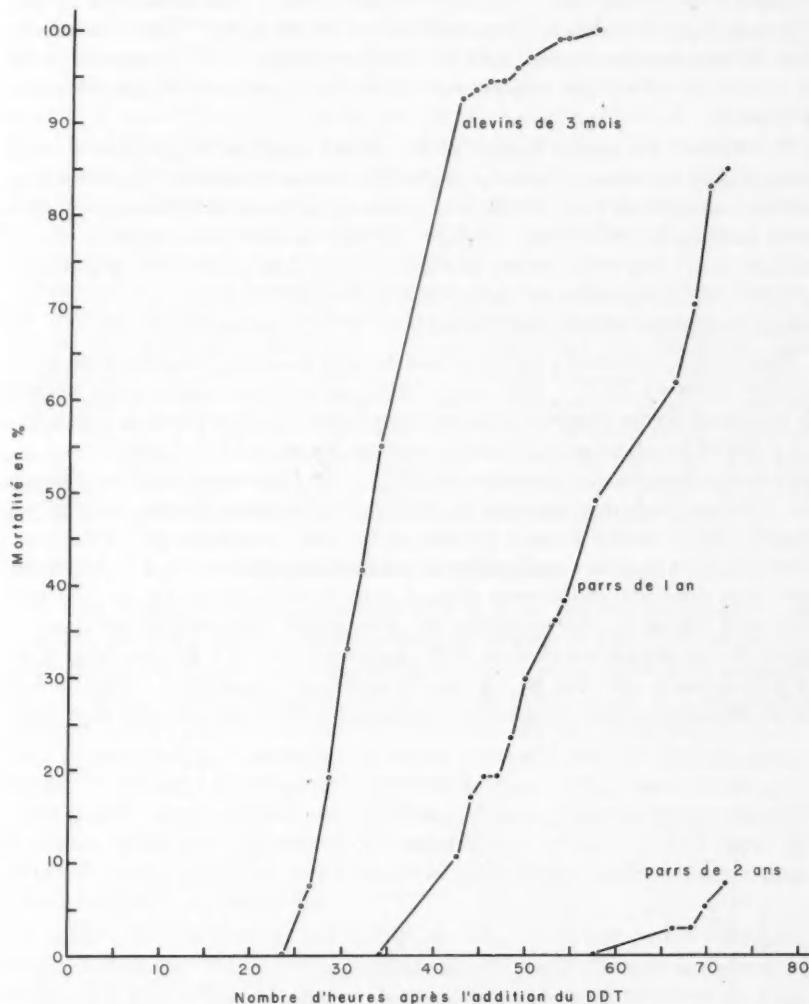
Fig. 2.   Courbes de mortalité chez des jeunes saumons: 94 alevins de 3 mois, 47 parrs de 1 an et 39 parrs de 2 ans.
    Concentration en DDT: 0.5 livre/acre (0.32 p.p.m.).
    Températures: 10.5°–16.5° C.
    Témoins: aucune mortalité enregistrée aux cours d'expériences effectuées dans les mêmes bassins, avec les mêmes nombres d'individus, alors que le Picco Hi-Solv seul fut substitué à la solution de DDT-Picco Hi-Solv.   Aucune expérience témoin fut effectuée chez des parrs de 2 ans.

Les résultats de la figure 2 démontrent que chez *S. salar*, la sensibilité est d'autant plus grande que l'individu est plus jeune.   D'ailleurs, c'est chose déjà connue pour quelques autres espèces de poissons (3, 6).   Chez le saumon, il est évident que les alevins, âgés de 3 mois, sont plus sensibles que les parrs de 1 an.   De même, ces derniers sont beaucoup plus sensibles que les parrs de 2 ans.

En utilisant les mêmes bassins et les mêmes nombres de spécimens, nous avons réalisé une série d'expériences chez les alevins de saumon, les alevins de truite et les parrs de 1 an, au cours desquelles nous avons remplacé les volumes de la solution de DDT-Picco Hi-Solv par des volumes identiques de Picco Hi-Solv seul.   Il n'y eut aucune mortalité, ce qui nous permet de conclure que la mortalité enregistrée au cours des expériences n'est pas attribuable au solvant organique utilisé pour dissoudre le DDT, mais bien au DDT.

Nous avons aussi étudié les variations du taux de mortalité en fonction de la concentration en DDT.   Pour cette étude, nous avons employé des alevins de saumon dans des bassins identiques mais dépourvus de système de pompage. Ce matériel fut choisi parce que nous savions, au moment de l'expérience, que ces alevins sont les plus sensibles au DDT.   Ce choix nous semblait logique car, en trouvant la dose maxima de DDT qu'ils pouvaient tolérer, nous étions assurés que les autres groupes étudiés ne seraient pas affectés par cette dose. Nous pouvons espérer l'application de ces faits expérimentaux, à la condition que cette dose maxima s'avère efficace dans la lutte contre les insectes que l'on veut détruire.   Les résultats de cette étude sont représentés dans la figure 3.   Ils démontrent que le DDT, à raison de 0.5 et 0.25 livre/acre (0.33 et 0.18 p.p.m.), tue tous les alevins de saumon.   Cependant, l'effet se fait sentir légèrement plus tard pour la concentration plus faible de 0.25 livre/acre.
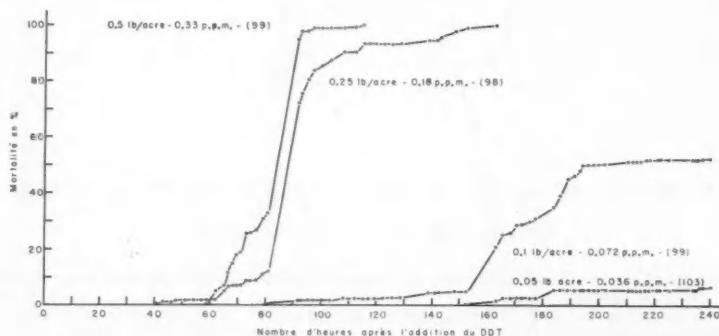

FIG. 3.   Courbes de mortalité chez les alevins de saumon.   Les chiffres, entre paren-thèses, indiquent le nombre d'individus utilisés dans chacune des expériences.
Concentration en DDT: telle qu'indiquée pour chacune des courbes.
Températures: 9.5°–20.0° C.
Témoins: aucune mortalité enregistrée chez les 99 alevins en eau pure.
Absence de circulation, par système de pompage, dans ces expériences.

Il fallait s'y attendre. Par contre, nous n'avons enregistré que 52% de mortalité par suite de l'emploi du DDT à raison de 0.1 livre/acre (0.072 p.p.m.). À ce sujet, il faut remarquer que 153 heures après le début de l'expérience, le taux de mortalité ne s'élevait qu'à 5%. Enfin, une concentration de 0.05 livre/acre (0.036 p.p.m.) ne produisit la mortalité qu'après 153 heures, et celle-ci n'atteignit que 7% après une période expérimentale de 240 heures. Pendant cette même période, nous n'avons perdu aucun des 99 alevins témoins.

À cause des volumes assez considérables d'eau et de solution employés, il nous était impossible de songer à maintenir ceux-ci à une température constante. Ils suivaient donc les variations de la température de l'air et nous avons ainsi enregistré des fluctuations parfois assez grandes. Les températures, dans les limites desquelles les expériences ont été réalisées, sont indiquées sur chacune des figures. Malgré ces variations de la température, les résultats ont pu être reproduits assez exactement comme le fait voir une étude comparative des courbes des figures 1 et 2. Les dosages de l'oxygène dissous selon la méthode de Nicloux nous ont révélé que les pourcentages de saturation ont varié entre 75 et 95%, cette dernière valeur étant obtenue immédiatement après une période de circulation forcée, par système de pompage, telle que décrite précédemment. Le pH de l'eau était toujours au voisinage de 7. La présence de la solution de DDT dans l'eau n'affectait pas cette valeur.

Discussion et Conclusions

Si nous comparons dans les figures 1, 2 et 3 les courbes représentant les résultats obtenus chez les alevins de saumon lorsque le DDT fut employé à raison de 0.5 livre/acre, nous voyons que la courbe de la figure 3 est déplacée vers la droite, c'est-à-dire qu'il a fallu plus de temps avant que les alevins meurent. Nous attribuons cette différence au fait que, dans ce cas, il n'y avait pas de système de pompage qui, comme nous l'avons dit précédemment, assurait le mélange plus rapide du DDT et de l'eau. Or, il est évident qu'il fallait plus de temps avant que les poissons ne fussent exposés à l'agent toxique. D'ailleurs, Everhart et Hassler (1) ont observé que la toxicité de la solution de DDT est plus grande pour les alevins de la truite brune, *Salmo trutta*, lorsque la solution est agitée dans l'eau.

Il ressort de nos résultats que l'usage du DDT, à raison de 0.5 livre/acre (0.32 p.p.m.), comporte de graves dangers pour les populations de saumon et de truite. En particulier, les alevins de ces deux espèces et les parrs de 1 an sont détruits, en presque totalité, dans les conditions expérimentales où nous avons opéré. Par contre, les parrs de 2 ans se sont montrés assez résistants pour nous laisser croire que le danger de mort par le DDT, à cette concentration, est chose peu probable. Rappelons toutefois que les alevins de truite et les parrs de 1 an se sont montrés plus résistants que les alevins de saumon, c'est-à-dire qu'il a fallu plus de temps pour atteindre une mortalité élevée chez ces groupes. Ces faits nous portent à croire que dans les conditions naturelles de milieu, où l'eau serait quelque peu renouvelée, un certain nombre

d'individus appartenant à ces catégories seraient peut-être épargnés.   Mais,
dans des considérations de ce genre, il ne faut pas oublier qu'en milieu na-
turel, il y a une foule de facteurs susceptibles de diminuer ou d'augmenter les
effets d'une vaporisation aérienne au DDT sur la vie aquatique.   Nous avons
été à même de constater l'un d'eux dans nos expériences.   Ainsi, les conditions
favorisant le mélange du DDT avec l'eau modifient grandement les effets
toxiques de celui-ci.   Comme corollaire de ceci, il est évident que dans les
études se rapportant aux effets du DDT sur la vie aquatique, il faut considérer
non seulement la quantité de DDT par unité de surface aqueuse (livre/acre),
mais aussi la quantité par unité de volume d'eau (p.p.m.).

Langford (6), se basant sur quelques observations trop peu nombreuses,
selon lui, croit que la concentration moyenne de DDT (D.L. 50) mortelle
pour l'alevin de *S. fontinalis* est inférieure à 0.001 p.p.m.   Nos résultats qu'il-
lustre la figure 3 nous laissent croire que cette valeur suggérée par Langford
est beaucoup trop faible.   Au contraire, ils nous indiquent qu'elle est plutôt
de l'ordre de 0.07 p.p.m.   Le fait que Langford ait utilisé une solution alco-
olique de DDT, produisant une suspension fine de DDT dans l'eau, peut pro-
bablement expliquer les différences entre ses résultats et les nôtres.

Très récemment, Hatch (2), utilisant 10 individus de chacune des espèces
suivantes: *S. salar*, *S. gairdneri* et *S. fontinalis* a trouvé, comme nous, que
les alevins de *S. salar* sont plus sensibles au DDT que ceux de *S. fontinalis*.
Cependant, les valeurs rapportées par Hatch sont très différentes des nôtres.
En effet, dans toutes ses expériences, la mort des sujets exposés au DDT
survient beaucoup plus tôt que nous l'ont montré nos propres observations,
et cela, même lorsque nous avons utilisé des concentrations plus grandes
(0.18 p.p.m. et 0.33 p.p.m.) que celle qu'il a utilisée (0.16 p.p.m.).   Bien que
les conditions dans lesquelles Hatch a opéré soient différentes des nôtres, il
demeure difficile d'expliquer ces divergences de résultats.   Toutefois, il con-
vient de souligner que Hatch n'a utilisé que 10 alevins.   Or, comme l'indiquent
nos résultats, la résistance au DDT n'est pas la même pour tous les individus,
et il est donc extrêmement difficile d'interpréter les résultats obtenus avec des
petits nombres d'individus.   Mentionnons aussi que Hatch a utilisé 30 alevins,
soit 10 individus appartenant à trois espèces différentes, dans 20 litres de solu-
tion, alors que nous avons utilisé 98 ou 99 alevins, dans 277 litres de solution.

Remerciements

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NEW RECORDS AND SPECIES OF CALANOID COPEPODS FROM SASKATCHEWAN AND LOUISIANA[1]

MILDRED STRATTON WILSON

Abstract

Diagnoses are given for three new species of the fresh-water calanoid copepod genus *Diaptomus* (subgenera *Hesperodiaptomus* and *Aglaodiaptomus*) from Saskatchewan and Louisiana. A check list of the calanoid copepods of Saskatchewan includes 23 species, of which 8 were previously reported in the literature, and 15 are new additions resulting from examination of collections from various types of water bodies.

Introduction

The present report includes brief diagnoses of three new diaptomid copepods found in fresh-water bodies of Saskatchewan in western Canada and Louisiana in southeastern United States. These two widely separated areas of North America are drawn together here because of the circumstance of finding, in Saskatchewan collections, an undescribed species which I had formerly known only from Louisiana. Over 240 samples from Saskatchewan, covering a wide range of water bodies from large, deep lakes to small temporary pools, have been examined for calanoid copepods. These records will be published in detail at a later date, and for the present, only a check list of known species is given.

Diagnoses of New Species

Diaptomus **breweri** sp. nov. (Fig. 1)

Synonymy

Diaptomus eiseni, Brewer 1898 (not Lilljeborg 1889), p. 128, Pl. 7, Figs. 9–12; in part, Marsh 1907, p. 481, Pl. 25, Fig. 3; 1918, p. 765, Fig. 1194a; 1929, p. 10, Fig. 1.

Diagnosis

Subgenus *Hesperodiaptomus*; *D. eiseni* group. Length, female 4.2–4.5 mm.; male 3.2–4.0 mm. Female metasomal wings not laterally produced or bifid; urosome three-segmented, genital segment without lateral protrusions, segment 2 about one-third length of segment 3; dorsum of caudal ramus as well as both margins covered with fine hairs. Female antennules and male left antennule with two setae on segment 11 and one seta on segments 13–19. Male right antennule, spine of segment 11 longer than that of 10 and subequal to that of 13; prominent spinous process at middle of segment 15 and at distal end of 16; segment 23 with strongly outcurved process that reaches to middle of apical segment or beyond. Leg 5 of female like that of *D. eiseni*, the setae of the third exopod segment well developed. Leg 5 of male much like that of *D. eiseni*, but differing in development of right second basal

segment. Anterior inner aspect of this segment very little expanded, its medial margin with a somewhat variable, narrow membranous protrusion with roughened edges, placed variably from the center third to the distal fourth of the segment; posterior face without ornamentation. Right exopod segment 2 without spinule on posterior face; outer lateral spine enlarged a little at base but strongly tapered and slender beyond the basal third, longer than width of segment. Claw nearly as long as exopod plus basal segment 2, strongly bent just above middle, the distal half irregular in outline and strongly incurved.

Specimens Examined

Three collections from Saskatchewan:

Type lot: four males, two females; prairie pool near Saskatoon; May, 1931; L. G. Saunders, collector. Holotype male, U. S. National Museum No. 101198; allotype female, No. 101199.

Four males; farm reservoir near Lucky Lake, highway No. 42; June 16, 1948; J. R. Nursall, collector. Occurring with *D.* (*Leptodiaptomus*) *nudus* Marsh and *D.* (*Aglaodiaptomus*) *saskatchewanensis* sp. nov.

Eight males, three females (one ovigerous); slough 1 mile northeast University of Saskatchewan campus, Saskatoon; May 8, 1957; E. B. Reed, collector. Occurring with *D.* (*Aglaodiaptomus*) *stagnalis* S. A. Forbes.

Remarks

Discovery of specimens of this form of the *D. eiseni* group clarifies the identity of Nebraska specimens described by Brewer (1). Presumably, a slide from Brewer's material was used by Marsh (4, 5) for illustration of the fifth leg of the male of *D. eiseni* Lilljeborg. Both Brewer's and Marsh's figures have puzzled me for a long time because the development of the protrusion of the second basal segment of the male right fifth leg did not seem to correspond with that observed in specimens of *D. eiseni* or the closely related *D. arcticus* Marsh. This discrepancy has also been noted by Kincaid (3), who referred to Marsh's figure as "misleading".

The type locality of *D. eiseni* is in central California, near Fresno. Specimens examined by me from that area, and considered to represent the type form, all have the inner anterior face of this segment expanded into a prominent flange, developed marginally toward its distal portion into a variable rugose or spiny protrusion. The medial part of the segment slopes upward from this flange to the posterior face, which is defined inwardly by a cuticular ridge and ornamented by a prominent, variable sclerotized structure (often a single, large spinous point). In *D. arcticus*, ornamentation of the posterior face is represented by the ridge and a very reduced spinous process, sometimes difficult to separate from the ridge with which it is usually closely associated. In any of these three forms, it is impossible to interpret correctly the actual structure of this complex segment unless unmounted appendages, not distorted by cover glass pressure, are studied from all possible aspects, preferably with both the stereoscopic and compound microscopes.

The narrow membranous protrusion on the inner part of this segment in *D. breweri* appears to correspond to the ornamented protrusion of the anteriorly expanded flange of *D. eiseni*. Otherwise the segment differs substantially. The anterior aspect is not expanded inwardly into a prominent flange, there is little medial swelling, and no sclerotized structure ornamenting the posterior face. In some specimens, there are indistinct or incomplete lines suggestive of remnants of the ridge, as indicated in Fig. 1. In other respects the leg is very similar to that of *D. eiseni*, as are the male right antennule and the female fifth leg.

The known distribution of *D. breweri* in Nebraska and Saskatchewan suggests that it may be a prairie form of the *D. eiseni* group.

Diaptomus dilobatus sp. nov. (Fig. 2)

Diagnosis

Subgenus *Aglaodiaptomus*. Length, female 1.8–1.87 mm.; male 1.65–1.7 mm. Female metasomal segment 5 with or without a dorsal cuticular protuberance, various in size, and asymmetrically placed on right side; metasomal wings asymmetrical, the left with large inner lobe that reaches beyond the posterior outer margin of the wing in dorsal view, the right wing with inner lobe hardly developed. Urosome of female three-segmented; proximal half of genital segment with lateral protrusions, the right side further
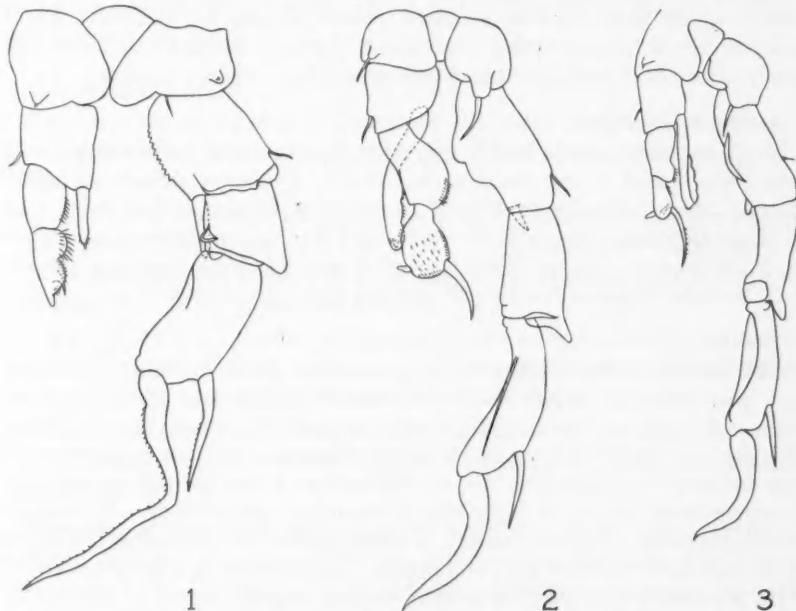
FIGS. 1–3. Leg 5 of male, posterior aspect. FIG. 1. *Diaptomus breweri* sp. nov. FIG. 2. *Diaptomus dilobatus* sp. nov. FIG. 3. *Diaptomus saskatchewanensis* sp. nov.

expanded into a medial, somewhat ventrally directed flange aligned with a distally placed, small, lobed process; caudal rami with hairs on inner margins. Female antennules and male left antennule with two setae on segment 11 and one seta on segments 13–19; setae of segments 17, 19, 20, and 22 with stiffly hooked ends. Male right antennule, length of spines of segments 10 and 11 less than that of 13; spinous processes present on segments 15 and 16; apical part of segment 23 with short outcurved process not reaching beyond middle of segment 24. Leg 5 of female of characteristic aglaodiaptomid structure with exopod segment 3 not developed but its two setae present. and the setae of endopod plumose and with enlarged bases; seta of exopod segment 2 present; endopod reaching beyond exopod 1.

Leg 5 of male (Fig. 2): Right leg with middle portion of inner margin of basal segment 2 expanded into flange with two major protrusions, usually developed as well-rounded lobes; exopod segment 1, outer distal corner broadly expanded and outwardly developed into a prominent, distally directed process; exopod 2 with medial ridge, the lateral spine placed at about distal fourth of segment; claw stout, its length subequal to that of exopod 2. Left leg reaching to middle of right exopod 1 or beyond; basal segments both short, the second only a little longer than the first; exopod 1 a little longer than exopod 2, swollen medially; exopod segment 2 constricted basally, the rest of segment expanded and rounded; both processes subterminal in position but widely separated by expansion of segment, the distal process directed outwards, the inner process setiform, about as long as segment. Right endopod reduced, not reaching to proximal fourth of exopod 1; left endopod nearly as long as exopod, its inner margin grooved and slightly crenate.

Specimens Examined

Numerous specimens of both sexes, three separate collections from a vernal pond near Grand Ecore, Natchitoches Parish, Louisiana; March 23, 1954; May 31, 1954: February 28, 1956; J. E. Sublette, collector. Occurring with *D. (Leptodiaptomus) moorei* M. S. Wilson and *D. (Aglaodiaptomus) clavipoides* M. S. Wilson. Type lot: collection of March 23, 1954; holotype female, U. S. National Museum No. 101202; allotype male, No. 101203.

Remarks

This species is closely allied to *D. marshianus* M. S. Wilson (14), known only from lakes and ponds in the vicinity of Tallahassee, Florida. These two are the only known aglaodiaptomids in which the female has a cuticular protuberance of the dorsum of the fifth metasomal segment. The females may be chiefly distinguished by the differences in the genital segment; in *D. marshianus*, the entire right side is expanded and partially overlies the second segment. The relationship of these species is indicated in the males by the similarity of their right antennules, the presence in the right fifth leg of the prominent outer distal process of the first exopod segment, and modification of the second basal segment by medial expansion rather than by ornamentation such as found in the related *D. conipedatus* Marsh. *D. dilobatus*

differs from *D. marshianus* and from all other aglaodiaptomids in the unusual medial lobed flange of the right second basal segment, and the shape of the left second exopod segment of the male fifth leg.

Diaptomus **saskatchewanensis** sp. nov. (Fig. 3)

Diagnosis

Subgenus *Aglaodiaptomus*. Length, Saskatchewan specimens: female 1.26–1.43 mm.; male, 1.18–1.22 mm.; Louisiana specimens: female 1.62–1.79 mm.; male 1.5 mm. Female metasomal wings asymmetrical, the left with large inner lobe that reaches beyond the posterior margin of the wing in dorsal view. Urosome of female three-segmented, the genital segment proximally with slight lateral protrusions; caudal rami with hairs on inner margins. Antennules and leg 5 of female similar to those of *D. dilobatus*. Leg 5 of male (Fig. 3): Right leg having basal segment 2 ornamented only with narrow, inwardly rounded, hyaline membrane on inner posterior face; exopod 1 nearly as long as basal segment 2, its outer distal corner with prominent distally directed process; claw subequal to or little shorter than exopod 2. Left leg noticeably reduced, reaching a little beyond end of right second basal segment, basal portion longer than exopod (29:23). Exopod segment 2 subequal to exopod 1, processes placed subterminally on outer and inner margins of segment, the innermost a curved seta about as long as the segment. Right endopod very reduced; the left reaching to near end of exopod, inwardly grooved and crenate.

Specimens Examined

Numerous specimens of both sexes from collections made in farm reservoirs of southern Saskatchewan, by the Fisheries Branch, Department of Natural Resources, Saskatchewan:

Type lot: Orr's Lake (a reservoir built by "Ducks Unlimited" on the Orr farm), near Unity; August 27, 1952; F. M. Atton, collector. Occurring with *D. (Aglaodiaptomus) leptopus* S. A. Forbes. Holotype female, U. S. National Museum No. 101200; allotype male, No. 101201.

A shallow cattle reservoir at Cedoux, southeastern Saskatchewan; August 10, 1949; J. R. Nursall, collector. Occurring with *D. (Leptodiaptomus) nudus* Marsh and *D. (Leptodiaptomus) siciloides* Lilljeborg.

Farm reservoir near Lucky Lake, highway No. 2; June 16, 1948; J. R. Nursall, collector. Occurring with *D. nudus* and *D. (Hesperodiaptomus) breweri* sp. nov.

Numerous specimens of both sexes from shallow, muddy roadside pond, highway No. 20, 12 miles south of Natchitoches, Natchitoches Parish, Louisiana; April 10, 1953; W. G. Moore, collector. Occurring with *D. (Leptodiaptomus) moorei* M. S. Wilson and *D. (Skistodiaptomus) pallidus* Herrick.

Remarks

D. saskatchewanensis does not appear to be closely allied to any other species of the subgenus *Aglaodiaptomus*. Its relationship is with a broadly defined group of which *D. spatulocrenatus* Pearse and *D. conipedatus* Marsh were first described, and to which may be further assigned *D. marshianus* M. S. Wilson, *D. dilobatus* sp. nov., and the inadequately characterized *D. pseudosanguineus* Turner (10) from Missouri. The distributional range of *D. saskatchewanensis* includes the type locality of *D. pseudosanguineus*, since it is to be expected that it will be found in localities between Louisiana and Saskatchewan, presently a very sparsely collected region. I have compared in detail all of the species of this group with Turner's description, and have rejected the possibility of correctly identifying any of them with *D. pseudosanguineus*, the recognition of which rests largely upon the characters of the female genital segment.

The structural features of both sexes of *D. saskatchewanensis* are relatively simple. The metasomal wings of the female are asymmetrical, and the genital segment has slight lateral protrusions. These characters must serve to separate the females from those of *D. conipedatus*, which likewise lacks distinctive structural modifications of the genital segment such as found in all other species of the group. The male fifth leg is distinguished by the simple structure and armature of the right second basal segment and the relative shortness of the left leg.

Check List of Saskatchewan Calanoid Copepods

In the literature, there are records of eight species of calanoid copepods from Saskatchewan. These are:

> *Senecella calanoides*: Rawson (9)
> *Limnocalanus macrurus*: Rawson (9)
> *Diaptomus sicilis*: Marsh (4) (as *D. tenuicaudatus*, n. sp.); Huntsman (2); Willey (11, 12, 13); Moore (7) (as *D. tenuicaudatus*)
> *Diaptomus leptopus*: Marsh (6)
> *Diaptomus oregonensis*: Marsh (6); Moore (7)
> *Diaptomus siciloides*: Moore (7)
> *Diaptomus ashlandi*: Moore (7)
> *Diaptomus nevadensis*: Moore (7) (as *D. shoshone*); M. S. Wilson (14)

These are combined below with the additional species found in the Saskatchewan collections I have examined, to form a check list of the species now known to occur in the Province. The total number of 23 species represents the largest number reported from any Canadian province as well as from any of the provinces or states of the prairie region of the Continent. The species particularly common in the collections that I examined are marked with a plus sign (+), those found in only one or two collections with a minus sign (−).

>   *Senecella calanoides* Juday
> + *Limnocalanus macrurus* Sars
> − *Heterocope septentrionalis* Juday and Muttkowski
>   *Epischura lacustris* S. A. Forbes
>   *Epischura nevadensis* Lilljeborg
> − *Acanthodiaptomus denticornis* (Wierzejski)

— *Diaptomus (Hesperodiaptomus) arcticus* Marsh
         breweri M. S. Wilson
—   *kiseri* Kincaid
—   *novemdecimus* M. S. Wilson
         nevadensis Light
+ *Diaptomus (Aglaodiaptomus) leptopus* S. A. Forbes
         forbesi Light
         saskatchewanensis M. S. Wilson
—   *stagnalis* S. A. Forbes
+ *Diaptomus (Leptodiaptomus) sicilis* S. A. Forbes
         siciloides Lilljeborg
         ashlandi Marsh
         minutus Lilljeborg
+   *nudus* Marsh
—   *pribilofensis* Juday and Muttkowski
  Diaptomus (Onychodiaptomus) sanguineus S. A. Forbes
+ *Diaptomus (Skistodiaptomus) oregonensis* Lilljeborg

As has been pointed out by Rawson (8), several of these species are the characteristic copepods of large, deep North American lakes (*Senecella calanoides, Limnocalanus macrurus, Epischura lacustris, Diaptomus sicilis, D. ashlandi, D. minutus, D. oregonensis*). A new discovery is the frequent occurrence in the same sample from large, fresh-water lakes of Saskatchewan, of the two lacustrine species of *Epischura*, formerly classifiable roughly as eastern (*E. lacustris*) and western (*E. nevadensis*) representatives of the genus. Before sorting Saskatchewan collections, I had been aware of the co-occurrence of these two species in a Manitoban lake (South Indian Lake) from which specimens had been sent to me for verification by Dr. I. G. Arnason. These two species were also found to occur together in samples from Great Slave Lake, Northwest Territory.

Some of the species are recorded east of the Rocky Mountain area for the first time (*E. nevadensis, A. denticornis, D. nudus, D. forbesi, D. kiseri, D. novemdecimus*). These emphasize a distribution pattern noted in other western species that range eastward only in the northern part of the Continent (15).

Records of occurrence in North America of *Acanthodiaptomus denticornis*, a broadly distributed species in Eurasia, are of zoogeographical importance. This species has been reported from several areas of Alaska, from Yukon Territory, British Columbia, Alberta, and Wyoming (15). The single occurrence in Saskatchewan collections was in a forested area of the west central part of the Province (an oxbow lake near the Beaver River, south of Waterhen Lake, about latitude 54° N. and longitude 108° W.). The associated calanoids were *Diaptomus nudus* and *D. leptopus*.

It is worthy of note that several very large diaptomid copepods in the size range of 3–5 mm. occur, sometimes together, in small water bodies in the vicinity of Saskatoon. In addition to *D. breweri* listed above, there have been found *D. stagnalis, D. arcticus*, and *D. novemdecimus*. It is to be expected that these species will be found to occur throughout the prairie region in small, often temporary, water bodies.

So far as known, *Diaptomus nevadensis*, another very large diaptomid copepod, occurs in Saskatchewan as in other regions of the Continent, in

saline waters. This is the only North American calanoid that seems to occur almost exclusively in such waters. Saskatchewan specimens of this species are similar to those of North Dakota which, as pointed out (14), vary slightly from those of far western states. The name *Hesperodiaptomus dentipes* given to this North Dakota variation by Kincaid (3) is here assigned to the synonymy of *D. nevadensis*.

Acknowledgments

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THE EFFECT OF A MICROSPORIDIAN PARASITE ON THE DEVELOPMENT, REPRODUCTION, AND MORTALITY OF THE SPRUCE BUDWORM, CHORISTONEURA FUMIFERANA (CLEM.)[1]

H. M. THOMSON[2]

Abstract

Infection of the spruce budworm, *Choristoneura fumiferana* (Clem.), by the microsporidian parasite *Perezia fumiferanae* Thom. retards both larval and pupal development and reduces pupal weight, fecundity, and adult longevity. These effects are more pronounced among the female insects. There is no evidence that the parasite affects male fertility, mate choice, or the fertility of eggs produced. The parasite causes some mortality, most of which occurs before the fifth instar. Among larvae infected orally, mortality seems to be related to the size of the initial dose. Mortality occurs equally in both sexes. The development and survival of the first instar and overwintering second instar are not affected by the parasite. It is suggested that the parasite causes most of the observed results by reducing the insect's ability to assimilate its food. Mortality, however, is believed to be due to the destruction of the mid-gut or Malpighian tubules.

Introduction

Although the Microsporidia have been known since the time of Pasteur and well over 100 species have been described, little attention has been paid to the host–parasite relationships. In 1949 a microsporidian was found in field populations of the spruce budworm, *Choristoneura fumiferana* (Clem.) (1). As studies of the mortality factors affecting the spruce budworm were intensified and attempts were made to work out life tables for this insect (4), it became increasingly important to know more about this protozoan and its effect upon the host. The life cycle and taxonomy of this parasite, *Perezia fumiferanae* Thom., were described in 1955 (6). Some of the inherent characteristics of the parasite such as spore longevity, specificity, and modes of transmission which affect the incidence of the parasite in host populations were reported in 1958 (7). The present paper is a description of some of the effects of parasitization on the host.

Much of the material used in the following experiments was obtained from a budworm infestation in the Uxbridge Forest near Uxbridge, Ont. This population was approximately 40% infected by the parasite and provided an excellent source of naturally infected insects.

Effect of the Parasite on Host Development

During the course of several budworm rearings, it was noticed that the infected insects were not as vigorous as their non-infected counterparts. The

infected insects seemed to develop more slowly, the pupae were smaller, and the adults shorter-lived than non-infected insects.   To test these differences, the length of time required for larval and pupal development, the pupal weight, and adult longevity were measured.   Naturally infected insects were used throughout experiments in order that the results might, at least partially, parallel the effect of the disease under natural conditions.   As the sexes differ considerably in respect to the above characteristics, it was necessary to differentiate between males and females.

Larval Development

Overwintering larvae from the Uxbridge Forest were forced out in the laboratory and reared individually to pupation.   The number of days from emergence until pupation was recorded, and the resulting pupae were examined for the presence of the disease.   All larvae dying during the rearing period were discarded.   The results, therefore, are based on those insects with a light initial infection.   Presumably heavier infections would simply accentuate the differences between infected and non-infected insects.   The results are shown in Table I.   The mean lengths of the larval periods of the infected and non-infected insects were compared by the t test and the differences observed were found to be significant at the 1% level for both males and females. Apparently the parasite significantly retards the rate of larval development.

TABLE I

LENGTH OF LARVAL PERIOD OF INFECTED AND
NON-INFECTED INSECTS

Status of insects	No. insects	Mean no. days
Males		
Infected	9	32.9
Non-infected	14	26.3
Females		
Infected	11	38.9
Non-infected	20	30.4

Pupal Development

Late-instar larvae from the Uxbridge Forest were allowed to pupate.   The length of time elapsing between pupation and adult eclosion, the state of health, and the sex of each insect were recorded and are shown in Table II.

TABLE II

LENGTH OF PUPAL PERIOD OF INFECTED AND
NON-INFECTED INSECTS

Status of insects	No. insects	Mean no. days
Males		
Infected	17	8.6
Non-infected	33	8.1
Females		
Infected	13	8.6
Non-infected	28	7.9

The difference between the pupal periods of infected and non-infected female insects is significant at the 1% level, and of the males at the 5% level. The parasite evidently retards pupal development.

Sequence of Adult Emergence

From the foregoing laboratory experiments, it is evident that infected insects develop more slowly both as larvae and as pupae than do non-infected insects. If insects under natural conditions are affected in a similar manner, non-infected adults should emerge in the field before infected adults. It would be reasonable to expect some overlap in emergence as very slightly infected insects would not be significantly retarded, and some non-infected insects might be retarded for reasons other than infection by *P. fumiferanae*.

To determine whether non-infected adults emerge first, mating pairs of budworms were collected in the field for 6 consecutive days, during which the bulk of the population emerged. Mating pairs were chosen because it was reasonable to believe that such insects had recently emerged, whereas single insects might have been at any age. The insects were examined microscopically for the presence of the microsporidian. The results, shown in Table III, demonstrate that the portion of mating adults infected by the parasite increased each day. The evidence from laboratory experiments that the developmental rate of infected insects is retarded is thus substantiated.

TABLE III

PRESENCE OF THE PARASITE IN MATING PAIRS COLLECTED
ON CONSECUTIVE DAYS

Day	No. insects	No. infected	% infected
1	80	9	11.3
2	108	19	17.6
3	70	19	27.1
4	70	22	31.4
5	78	38	48.7
6	188	93	49.5

Overwintering Stages

Also considered was the possibility that the parasite might retard development during the overwintering stages. If this were the case the infected insects would emerge later than the non-infected insects in the spring. To test this possibility, overwintering insects from the Uxbridge Forest were forced out in the laboratory early in the spring of 1957. The sequence of emergence was recorded and the insects were examined for the presence of the parasite. From the results of this study (Fig. 1), it is evident that the emergence of the infected overwintering larvae was not significantly retarded in comparison with the non-infected insects.

Pupal Weight

Two groups of fourth-instar larvae were reared in the laboratory: one group was supplied with food sprayed with *P. fumiferanae* spores, the other group
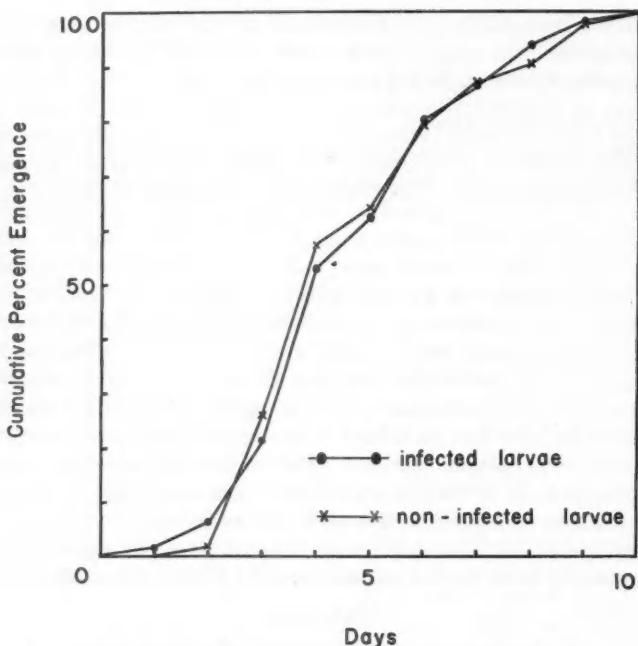
FIG. 1. Cumulative percentage emergence of infected and non-infected second-instar budworm larvae.

received food sprayed with distilled water. As pupae were formed, they were weighed, sexed, and checked for the presence of the parasite. The results are shown in Table IV. A *t* test indicated that the difference between weights of diseased and non-diseased pupae is significant at the 1% level for both sexes. In this case the disease exerted a strong effect on the pupal weight, reducing the weight of female pupae by almost 50%. However, these insects were artificially infected and as a consequence developed extremely heavy infections. To determine whether naturally infected insects are similarly affected, the pupae from the pupal development experiment were weighed. These

TABLE IV

THE EFFECT OF THE PARASITE ON PUPAL WEIGHT (MG.)

Status of insects	No. insects	Mean weight
Males		
Infected	9	35.4
Non-infected	16	54.1
Females		
Infected	7	52.5
Non-infected	6	98.3

insects had been removed from their natural environment for only a short time and had received their infections in the field. These results are shown in Table V. A t test indicates that the difference between the infected and non-infected female pupae is significant at the 1% level. There is no significant difference between the male pupal weights. Thus under the lighter degree of infection found in nature, the disease reduces the female pupal weight by approximately 25% but has little or no effect on male pupal weight.

TABLE V

THE EFFECT OF THE PARASITE ON THE PUPAL WEIGHT OF NATURALLY
INFECTED INSECTS (MG.)

Status of insects	No. insects	Mean weight
Males		
Infected	17	58.3
Non-infected	33	57.2
Females		
Infected	13	78.3
Non-infected	28	101.7

Adult Longevity

The length of life of infected and non-infected adults was determined from the same material used to ascertain pupal development. The adults were kept in individual vials and the time elapsing between adult eclosion and death was measured. The results are shown in Table VI. A t test indicates that

TABLE VI

THE EFFECT OF THE PARASITE ON ADULT LONGEVITY

Status of insects	No. insects	Total days	Mean no. days
Males			
Infected	17	122	7.2
Non-infected	33	320	9.6
Females			
Infected	13	114	8.7
Non-infected	28	442	15.8

the differences between the infected and non-infected insects of both sexes are significant at the 1% level. Both non-infected males and the non-infected females live longer in the adult stage than their infected counterparts, although the effect is much more pronounced in female insects. It should be noted that the adults probably live a much shorter time under natural conditions. The adults in vials are rather quiescent and hence their available store of energy in the fat body is expended slowly. An adult in nature, which actively crawls and flies and has to contend with wind and rain, would burn energy stores at a much faster rate. The effect of infection under these conditions might be accentuated.

From the foregoing experiments, infection by *P. fumiferanae* retards the development of the feeding larvae and the pupae, reduces pupal weight, and shortens the adult life span.   These effects are much more pronounced among the female insects.   The parasite, however, does not affect the development of the overwintering larval stage.

Effect of the Parasite on Reproduction

To determine whether the parasite affects mate selection, egg production, or egg viability, a total of 134 copulating pairs was collected from the Uxbridge Forest population.   They were all collected on the same day, to increase the uniformity of the sample, and the day selected was near the end of the flight period in order to ensure a high percentage of infected individuals.   Each pair was placed in a small container with a twig of spruce foliage.   The females oviposited on the foliage during a period of a week to 10 days and then died; the males died soon after mating.   The dead adults were examined for the presence of the parasite and the number and fertility of the eggs was determined by examination of the hatched egg masses.

Mate Selection

The 134 matings fell into four groups: non-infected males × non-infected females, non-infected males × infected females, infected males × non-infected females, and infected males × infected females.   The number of matings in each group were compared with those expected on a random basis.   The results are shown in Table VII.   A chi-square test indicates that the number of matings of each type does not differ significantly from those expected on a purely random basis.   There is, therefore, no evidence that infection exerts any influence on the mate choice of the adult insects.

TABLE VII

COMPARISON OF ACTUAL AND EXPECTED NUMBER OF MATINGS OF VARIOUS
COMBINATIONS OF NON-INFECTED AND INFECTED ADULTS

	Males		
Females	Non-infected	Infected	Total
Non-infected	40 (37.5)*	28 (30.5)	68
Infected	34 (36.5)	32 (29.5)	66
Total	74	60	134

*Numbers in parentheses are those expected on a random basis.

Number of Eggs

Campbell (3) has shown strong positive correlation between female pupal weight and fecundity.   On this basis and the results of an earlier experiment, which showed that infected pupae were lighter than their non-infected counterparts, it was expected that infected females would produce fewer eggs.

TABLE VIII

MEAN NUMBER OF EGGS RESULTING FROM MATINGS OF VARIOUS COMBINATIONS
OF NON-INFECTED AND INFECTED ADULTS

| | Males | | |
Females	Non-infected	Infected	Mean
Non-infected	168.9	170.3	169.5
Infected	156.6	153.4	155.0
Mean	163.2	161.3	

The number of eggs resulting from the 134 mated females, some infected and some non-infected, were used to determine the effect of the parasite on egg production. The results, shown in Table VIII, indicate that there is very little difference between the mean number of eggs produced by females mated to infected and non-infected males and a t test indicates that this difference is not significant. Infection in the male, therefore, has no effect on the number of eggs produced by its mate. A t test on the difference between the mean number of eggs produced by infected and non-infected females, however, indicates that these figures are significant at the 1% level. It is apparent that the parasite does reduce the number of eggs produced by infected females; the reduction in this case amounting to 8–9%. It should be noted that, on the basis of egg production, the Uxbridge population is very vigorous; it is possible that the parasite might be of greater effect in less vigorous populations.

Egg Sterility

To determine whether the microsporidian infection might cause egg sterility, the numbers of sterile eggs produced by each of the four previously described mating groups were counted. The results are presented in Table IX. A t test as applied to percentage indicates that the difference between the percentages of sterile eggs produced by the above matings is not significant. It is, therefore, concluded that the infection does not influence the fertility of the eggs produced.

TABLE IX

EGG STERILITY RESULTING FROM VARIOUS COMBINATIONS OF NON-INFECTED
AND INFECTED ADULTS

Mating type	No. families	No. eggs	No. sterile eggs	% sterile eggs
Non-infected male ×				
(1) non-infected female	40	6757	366	5.4
(2) infected female	34	5323	349	6.5
Infected male ×				
(1) non-infected female	28	4768	231	4.8
(2) infected female	32	4910	350	7.1

Mortality

Mortality at Natural Levels of Infection

The following experiments were carried out to determine the effect of the parasite on mortality among congenitally infected larvae. Larvae from Uxbridge Forest were reared individually from time of emergence from the hibernacula until death. Mortality records were kept and all larvae were examined after death to determine whether the parasite was present. Thus, each rearing could be divided into two groups, one infected by *P. fumiferanae*, the other non-infected, the latter group serving as a control. As these larvae were collected in the field, many mortality factors operated in addition to the microsporidian parasite, so that all mortality among diseased larvae cannot be attributed to the microsporidian. To complicate the situation further, the presence of the microsporidian in a dead insect is not a sure indication that death was caused by the parasite; this is evidenced by the fact that some adults develop from heavily infected larvae. Therefore, it is impossible to decide what fraction of this mortality can be attributed to *P. fumiferanae*. However, by comparing the mortalities of the infected and non-infected groups, the additional mortality caused by the microsporidian can be determined. Three rearings were conducted, two in 1956 and one in 1957; as these were conducted at different times and from different populations, they cannot be considered to be replicates. From the results shown in Table X, two facts are apparent:

TABLE X

PER CENT MORTALITY OF INFECTED AND NON-INFECTED BUDWORM

| | Rearing group No. | | |
	I	II	III
Infected larvae	81.3	84.3	79.7
Non-infected larvae	73.6	73.0	53.7

(*a*) Diseased larvae suffer heavier mortality than do non-diseased larvae. It follows, therefore, that the disease contributes to mortality at these natural levels of infection.

The rearing records indicate that most of the mortality among the diseased larvae occurs before the late fourth or early fifth instar. To determine whether this also is the case in nature, another rearing experiment was conducted. In this experiment the larvae were removed from the field in June, 1956, when the bulk of the population was in the late fourth instar. The mortality among these larvae was as follows:

<div align="center">

infected larvae 21.5%,
non-infected larvae 20.4%.

</div>

The additional mortality observed among the diseased larvae in earlier rearings is not present among these larvae. It follows that most mortality caused by the parasite occurs before the early fifth instar both in the laboratory and in the field.

(b) The parasite is not lethal to all the insects at the levels of infection occurring in the emerging larvae and some of the infected larvae survive to adulthood. This is important as it ensures passage of the parasite to the following generation regardless of the amount of intrageneration transmission (7). Thus in a low-density population where transmission from larva to larva is difficult, the following generation would be infected by the surviving diseased adults. Admittedly such transmission would not be effective indefinitely, but it would allow the parasite to survive unfavorable periods that might otherwise eliminate it from the population.

Mortality during the First Instar

Mortality during the first instar was measured as failure to form hibernacula. The larvae used were obtained from 20 families, in 10 of which the female parent was infected and in the other 10 the female was free from infection. The male parents were all free from infection by *P. fumiferanae*. The offspring of the infected females were assumed to be infected (7) and a microscopic examination of a random sample of these larvae revealed the presence of spores in all larvae sampled. Each family of larvae was placed in a Petri

TABLE XI

MORTALITY DURING THE FIRST INSTAR

	Larvae forming hibernacula	Larvae not forming hibernacula	Total
Infected	404 (417)*	135 (122)	·539
Non-infected	417 (404)	105 (118)	522
Total	821	240	1061

*Numbers in parentheses are those expected on a random basis.

dish prepared in the manner described by Stehr (5). The larvae formed hibernacula which were subsequently counted; the percentage of larvae failing to form hibernacula was calculated. The data are given in Table XI. The experimental chi-square is greater than the 5% level of significance; therefore, there is no evidence that the parasite causes mortality during the first instar.

Mortality among Overwintering Second-instar Larvae

Mortality during the overwintering portion of the second instar was measured as failure to emerge from the hibernacula after suitable temperature treatment. The larvae used in this experiment were taken from 40 families, half of which were infected. The hibernating larvae were maintained for 4 months at 0° C. and then transferred to 21° C. for 2 weeks. At the end of the time the number of larvae that had emerged from each family were counted. The percentages of infected and non-infected larvae failing to emerge from the hibernacula are shown in Table XII.

It should be pointed out that 80% mortality is abnormally high and probably reflects unfavorable rearing conditions. However, as there is no

TABLE XII

MORTALITY AMONG OVERWINTERING LARVAE

Health	Total hibernacula	Total mortality	Per cent mortality
Infected	806	647	79.5
Non-infected	801	640	80.0

significant difference between the mortality of infected and non-infected insects, it is assumed that the parasite does not cause mortality during the overwintering period.

The Effect of the Size of the Initial Infection

To determine whether infection and mortality were affected by the size of the initial dose, the following experiment was conducted. Eighty larvae from a relatively disease-free area of Ontario were divided into four groups of 20 larvae. One group was fed foliage sprayed with distilled water, the other groups were fed foliage sprayed with spore suspensions of 10×10^6, 30×10^6, and 100×10^6 spores per ml., respectively. The insects were allowed to feed on the sprayed foliage for 24 hours and then were reared individually on clean foliage. It should be emphasized that only a small fraction of the spores was consumed; nevertheless, the larvae in group III should have received approximately 10 times as many spores as the larvae in group I. The results, shown in Table XIII, indicate that all the test larvae became infected with the parasite regardless of the spore dosage but that mortality was related to the size of the initial dose.

TABLE XIII

THE EFFECT OF THE SIZE OF THE INITIAL DOSE ON MORTALITY

Group; spores/ml.	No. larvae	Mortality when examined on:				Total
		15th day	20th day	25th day	30th day	
I 10 million	20	0	2	1	0	3
II 30 million	20	4	2	2	0	8
III 100 million	20	6	4	4	2	16
IV Control	20	0	1 *	0	0	1

*Not infected with Microsporidia.

Mortality in Relation to Sex

During the course of several experiments it was noticed that the effects of the disease were more pronounced among the female insects. To determine directly whether mortality also is greater among female insects is rather difficult as many deaths occur before the sex of the larvae becomes apparent. However, some information may be gained by indirect methods.

If it may be assumed that both sexes are equally susceptible to infection, and there is no indication otherwise, then any difference between the mortality of immature males and females should result in a difference between the percentages of male and female adults that are infected. Increased mortality among female larvae should result in a decreased percentage of infected female adults.

In one rearing experiment 34% of the adult males and 31.7% of the adult females were infected. Again in a collection of 134 mating pairs in the field 44.8% of the males and 49.2% of the females were infected. These observations do not seem to indicate any consistent differential mortality between the sexes.

Discussion

A summary of the effects of parasitization of the spruce budworm by *P. fumiferanae* is given in Table XIV. It is apparent that the parasite causes a debilitating disease. Most such diseases result from depriving the host of energy. This may be done directly by breaking down host tissues or indirectly by interfering with the host's ability to obtain and assimilate its food. In the case under discussion it is believed that the parasite acts in both ways. In a heavily infected larva spores may make up 25% of the host volume; as the parasites develop within the host cells, all the material and energy required to form these spores is obtained directly at the expense of the host. However, it is believed that all the principal effects of infection are caused by a reduction in the host's ability to assimilate its food.

TABLE XIV

SUMMARY OF EFFECTS OF THE PARASITE ON THE BUDWORM

Host stages	Effects of the parasite
Eggs	No effect
Instar I	No effect
Hibernating II instar	No effect
Instar II–IV	Some mortality; retards development
Instar V–VI	Retards development and has general debilitating effect; little mortality
Pupae	Slightly retards development; reduced weight of females; little mortality
Moths	Emergence delayed; shortened adult life, especially in females; may cause some mortality, i.e. death before mating; reduces fecundity

The primary site of infection is the mid-gut (6); this organ has many important functions including digestion and absorption of food. It is reasonable to believe that cells tightly packed with spores must function very inefficiently, if at all. Thus, in infected larvae there is a reduction in the functional area of the mid-gut, the amount of reduction, of course, depending upon the degree of infection. Infected larvae will be less efficient at forming nutrient reserves

for the pupal and adult stages—one of the principal functions of the larvae. This is supported by the observations that the fat bodies of infected larvae are smaller than those of non-infected larvae and the weight of infected larvae is smaller than that of non-infected larvae and the weight of infected pupae is less than that of non-infected pupae. On this basis, the effects of the disease are, for the most part, what one would expect.

It might be expected that infected larvae would simply develop into smaller pupae without being retarded in the process. In this connection it is well to remember that the processes of molting and pupation are under hormonal control and in most, if not all, insects can occur only after the animal has accumulated a certain nutritive reserve (2). Infected larvae will require more time to build up this reserve and will, therefore, develop at a slower rate.

As infected larvae cannot build up large fat reserves, the resulting pupae will be lighter. The difference between non-infected and infected pupae is exaggerated by the fact that a large portion of the pupal weight is due to the fat stores. Concomitantly the rate of development to the adult stage is slower in infected pupae.

As the budworm adult is entirely dependent upon stored food as a source of energy, it follows that infected adults will not live as long as non-infected adults. This effect is much more noticeable in female moths as they convert a large portion of their reserves into yolk in the formation of eggs. It then follows that a diminished number of eggs is also a logical result of infection.

Thus the principal effects of infection may be attributed to a reduction in the efficiency of the mid-gut to assimilate food. However, some insects are dilled by infection. The dosage experiments indicate that mortality varies directly with the size of the initial infection; larvae, if heavily enough infected, will die. But what is the mechanism of death? If mortality results simply from an even greater reduction in the ability of the mid-gut to assimilate food, then the dead larvae should exhibit signs of starvation. This, however, is not the case: although the dead larvae are retarded in development, they do not exhibit the shrunken appearance associated with starvation. Therefore, mortality is not a result of an extension of ailments of the sick larvae but rather is due to other factors.

Comparison of the tissues of infected, but active, larvae and those of moribund larvae reveals several important differences. The tissues of the moribund larvae are, of course, more heavily infected; however, the most striking differences are seen in the mid-gut and the Malpighian tubules. The mid-gut of a moribund larva shows a general breakdown: the cells are loosened from each other and from the basement membrane, some have disintegrated, and clumps of cells may be found floating in the lumen of the gut. It is reasonable to believe that the infection reaches a point where cells are being destroyed. The lumen of the Malpighian tubules of moribund larvae is frequently so clogged with spores that it is difficult to believe that the organ could function. As both the mid-gut and the Malpighian tubules are extremely important organs with many vital functions, it is clear that either of the above-described conditions could bring about the death of the insect.

Thus the light and medium infections simply lower the efficiency of the insect, whereas the heavy infection destroys or mechanically prevents the function of certain organs, resulting in the death of the insect.

Experiments have shown that the parasite does not cause mortality of the host during the egg stage, the first instar, or the hibernation period of the second instar; furthermore there is evidence that the parasite does not retard the development of these stages. These findings are not surprising in view of previous observations (7) that during the above stages the spores are suspended in the yolk material and are not actively parasitizing the host tissues. Infection of the mid-gut occurs sometime near spring emergence; presumably as the yolk material is utilized conditions within the mid-gut become favorable for spore germination.

While *P. fumiferanae* causes some direct mortality of its budworm hosts under laboratory conditions, this mortality may be considerably increased in nature by combination of the disease with other causes of mortality. The prolonged larval development and the generally debilitated condition of the diseased insects enhance the potentialities of these other factors. Similarly, the oviposition of diseased females would be further reduced in nature where the female insect must use a portion of her fat stores for general activity. As the diseased female has smaller food reserves, a proportionally larger amount of these stores would be diverted from egg formation. Under natural conditions considerable mortality, direct or indirect, may occur among infected larvae.

Acknowledgments

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SOME BIOLOGICAL REQUIREMENTS AND HOST–PARASITE RELATIONS OF ENTAMOEBA INVADENS[1]

EUGENE MEEROVITCH

Abstract

A hypothesis explaining the processes leading to the encystation of *E. invadens* is presented. It is suggested that *E. invadens* can live in turtles as a harmless commensal because the conditions necessary for its encystation and the completion of its life cycle are available in the intestinal lumen of turtles. As these conditions are not present in snakes, the amoebae feed on the mucous secretion of the snakes' intestinal epithelium, and invade the tissues.

Following the writer's hypothesis that turtles are the natural hosts of *Entamoeba invadens* and that snakes may only become accidental hosts of this parasite (12), it was decided to try to elucidate the factors which determine extreme pathogenicity of this parasite in snakes, and its ability to live in the turtles as a harmless commensal.

In an ideal case of parasitism (the term here being used in its broad sense to include the condition known as commensalism), the interplay of host–parasite relations must be such as to enable the parasite to propagate its species, and to create conditions whereby neither the host nor the parasite becomes the loser. When this principle is applied to amoebic parasitism, then a satisfactory condition may be said to exist when the amoebae are able to encyst in the lumen of the host's intestine and pass out to the exterior, and when they do not invade the host's tissues, imperiling the well-being or even the life of the latter.

It appeared therefore that a study of requirements for the encystation of *E. invadens* in vitro might elucidate the factor or factors, either present or absent in the chelonian and the ophidian hosts, which determine the ability of the amoeba to live commensally, that is, to complete its life cycle in the lumen of the host's intestine. Some parallel observations were also made on *Entamoeba terrapinae*, another non-pathogenic parasitic amoeba of turtles.

Materials and Methods

The strains of *E. invadens* used in this work were:

1. the P.Z. strain, isolated from a snake which died at the Philadelphia Zoo, and maintained in vitro at the Institute of Parasitology since 1947;

2. the I.P.-1 strain, isolated at this Institute in 1952 from *Natrix cyclopion*, which was imported from Florida;

3. the I.P.-2 strain, isolated from a painted turtle, *Chrysemys picta* (12).

The strain of *E. terrapinae* was isolated also from another *Chrysemys picta*.

   E. invadens and *E. terrapinae* were maintained in vitro at room temperature
in mixed cultures (containing the concomitant bacterial flora), in serial trans-
fers every 2 weeks in Balamuth's buffered egg-yolk infusion medium with
liver extract and rice starch (2).

   The bacteria-free cultures of *E. invadens* were maintained according to
the method of Miller (13).   A modification of this technique consisted in the
bleeding of the hamsters to death by means of cardiac puncture prior to the
excision of the liver, in order to avoid as much as possible the introduction of
whole blood into the cultures.   Penicillin in the concentration of 1000–2000
I.U. per ml. of medium and streptomycin in the concentration of 1000–2000
μg. per ml. of medium were added to the bacteria-free cultures.   The bacteria-
free cultures were sealed with a plug of sterile petroleum jelly and were kept
at room temperature with serial transfers every 4 to 5 weeks.

   The apparatus used in "double-culture" experiments is shown in Fig. 1.
The mixed cultures of amoebae and bacteria or cultures of bacteria alone were
grown in Balamuth's medium in the outer tubes, while bacteria-free cultures
of amoebae were grown in the inner cellophane tubes in the S–S–A (saline–
serum–antibiotics) medium with a piece of hamster liver, which rested on the
convex glass surface at the lower end of the cellophane tubing.   Inoculation
into the outer tubes was made through the side arms, while the bacteria-free
amoebae and other additions were introduced into the S–S–A medium through

liquid
paraffin

cellophane
tubing

liver

Balamuth's
medium

S-S-A medium
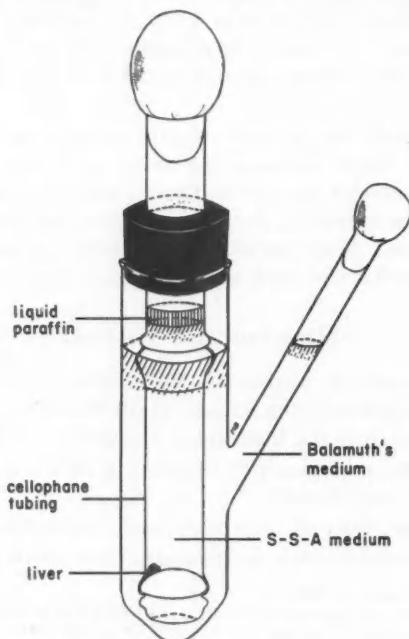
FIG. 1.   Double-culture apparatus.

the top opening, which was plugged with cotton. Cellophane tubing was sealed to glass by means of household cement,* which made the junctions impermeable to bacteria. The apparatus, with Balamuth's medium in the outer tubes, was sterilized by autoclavization; the S–S–A medium and liver were introduced with sterile precautions into the inner cellophane tubes when the apparatus had cooled to room temperature. The bacteria-free cultures in the cellophane tubes were sealed with sterile liquid paraffin.

Glycogen was demonstrated in the amoebae and cysts by the use of Best's carmine stain. Fixed culture material was embedded in formaldehyde-coagulated serum clots, which were attached to microscope slides before staining.

Experimental

Mixed cultures of *E. invadens* and of *E. terrapinae* nearly always produced very heavy growths of trophozoites (up to several hundred per microscope field) and correspondingly large numbers of cysts. As the cultures become older (a period of two to four weeks after initiation), the number of trophozoites relative to that of the cysts became progressively smaller, until only cysts were seen in the cultures. The cysts did not excyst but remained viable for a long time, even after storage in the refrigerator at 4–5° C. for periods up to a month or more. The nature of the inoculum (the proportion of trophozoites and cysts to each other, or the total number of organisms in the inoculum) did not have any effect on the ultimate population density of a culture. With smaller numbers of organisms inoculated, it took somewhat longer for a culture to reach the peak of density.

The trophozoites ingested starch granules avidly. Contrary to the observations of McConnachie (11), mass encystation could not be related to the depletion of free starch in the medium. In some cultures where mass encystation had occurred, there was still plenty of free starch; in others, under seemingly identical conditions, the amount of free starch was reduced, or it was completely digested.

Some cultures, however, failed to produce cysts, or produced them in small numbers. Because the medium was buffered to pH 7.2–7.4, significant changes in hydrogen-ion concentration did not take place, and could not be held responsible for the failure of the amoebae to encyst.

Bacteria-free cultures of the three strains of *E. invadens* were never as rich as those containing bacteria. The peak of multiplication was usually reached two to three weeks after the cultures were initiated, but cysts were never found in any of the bacteria-free cultures. While some bacteria-free cultures remained positive for amoebae for periods up to six months, most became negative after eight to ten weeks. Most of the amoebae in the bacteria-free cultures aggregated at the bottom of the tube in the vicinity of the liver fragment. Autoclaved hamster and snake liver did not support the growth of bacteria-free amoebae. The addition of starch to bacteria-free cultures did not induce encystation, although the amoebae ingested starch granules.

*C-I-L Household Cement, manufactured by Canadian Industries Limited.

It was observed that the trophozoites of *E. invadens* and *E. terrapinae* ingested solid particles irrespective of their food value.   Thus, while the ingestion of starch in the mixed cultures was a general rule, the trophozoites ingested particles of charcoal or kaolin when no other solids were available. When indigestible solid particles were present in the cultures together with the starch, the amoebae seemed to have ingested only the latter.   This was probably due not to selective ingestion, but to the fact that the indigestible particles were eliminated, leaving only the starch granules within the amoebae.

Cannibalism (the ingestion of cysts), was often seen in *E. invadens* and *E. terrapinae*.   Lamy (7) also observed cannibalism in *E. invadens* and considered it unique because the amoebae ingested cysts of their own species.

The trophozoites of *E. invadens* in the bacteria-free cultures ingested liver cell debris; bacteria were readily ingested by *E. invadens* and *E. terrapinae*. The ingestion of erythrocytes by *E. invadens* was reported by Rodhain and Van Hoof (16).

The most striking difference in the behavior of *E. invadens* in mixed cultures and in bacteria-free cultures was that encystation did not take place in the latter.   Encystation in mixed cultures was not restricted to Balamuth's medium.   The amoebae encysted on other media used for the cultivation of *E. histolytica*, provided that rice starch or mucin particles and living bacteria were present.

The results of many trials where bacteria-free *E. invadens* was used as inoculum permitted the following conclusions:

1. when bacteria were present, but neither starch nor glycogen were added, there was a very feeble growth of the amoebae, with hardly any encystation taking place;

2. when bacteria-free *E. invadens* was inoculated into sterile Balamuth's medium without any additions, the amoebae soon died;

3. when glycogen and bacteria were added to the cultures, the results were the same as when only the bacteria were added, i.e. there was a weak growth but almost no encystation;

4. when glycogen was added in the absence of bacteria, the results were the same as in the pure medium, i.e. the amoebae soon died;

5. when starch was added in the absence of bacteria, the amoebae ingested it, but did not encyst and the cultures died out after 1 or 2 days.   The addition of glycogen to such cultures did not change the results at all.

It was clear, therefore, that bacteria and starch or mucin were two extrinsic factors without which mass encystation could not take place.

It was mentioned earlier that *E. invadens* never became as numerous in bacteria-free cultures as in the mixed ones.   Some mixed cultures failed to produce heavy growths of amoebae, and as a result, there was little or no encystation.   In other words, mass encystation could be correlated not only with the presence of bacteria and starch or mucin, but also with a preliminary abundant growth of trophozoites.   This observation confirmed those of other workers (4, 5).

It is common knowledge that the cysts of parasitic amoebae contain large accumulations of glycogen. A cytochemical study by means of staining with Best's carmine was made on glycogen accumulation in the trophozoites and cysts of *E. invadens* and *E. terrapinae* from mixed cultures and the trophozoites of *E. invadens* from bacteria-free cultures. Control preparations were treated with saliva prior to staining in order to ascertain the identity of the chromophilic substance as glycogen. The results of this study are summarized in Table I.

TABLE I

GLYCOGEN ACCUMULATION IN THE CULTURES OF *E. invadens* AND *E. terrapinae* AS EVIDENCED BY STAINING WITH BEST'S CARMINE

Amoebae			Age of culture, days	Population density		Glycogen accumulation	
Species	Strain	Kind of culture		Trophozoites	Cysts	In trophozoites	In cysts
E. terrapinae		Mixed	15	++++	+	Little or none	Heavy
E. terrapinae		Mixed	15	++++	+	Little or none	Heavy
E. terrapinae		Mixed	15	++++	−	None	
E. terrapinae		Mixed	15	++++	+	Little or none	Heavy
E. terrapinae		Mixed	15	++++	+	Little or none	Heavy
E. terrapinae		Mixed	15	++++	−	None	
E. terrapinae		Mixed	15	++++	−	None	
E. terrapinae		Mixed	14	++++	++++	Heavy	Heavy
E. terrapinae		Mixed	14	++++	−	Little or none	
E. terrapinae		Mixed	14	+++	−	None	
E. terrapinae		Mixed	14	+	++++	Heavy	Heavy
E. invadens	I.P.-1	Mixed	39	+++	+	Heavy	Heavy
E. invadens	I.P.-1	Mixed	14	−	+++		Heavy
E. invadens	I.P.-2	Mixed	15	++	++++	Heavy	Heavy
E. invadens	I.P.-2	Mixed	15	++++	++++	Heavy	Heavy
E. invadens	I.P.-2	Mixed	26	+++	−	None	
E. invadens	I.P.-2	Mixed	26	−	++++		Heavy
E. invadens	I.P.-2	Mixed	26	++++	++	Moderate	Heavy
E. invadens	P.Z.	Mixed	26	++++	−	None	
E. invadens	I.P.-1	Bacteria-free	30	+	−	Little in some	
E. invadens	I.P.-1	Bacteria-free	45	++	−	Little in some	
E. invadens	I.P.-1	Bacteria-free	45	++	−	Little in some	
E. invadens	I.P.-1	Bacteria-free	45	+	−	Little in some	
E. invadens	I.P.-1	Bacteria-free	45	+	−	Little in some	
E. invadens	I.P.-1	Bacteria-free	45	++	−	Little in some	
E. invadens	I.P.-1	Bacteria-free	10	+++	−	Little in some	
E. invadens	I.P.-2	Bacteria-free	10	+++	−	Little in some	
E. invadens	P.Z.	Bacteria-free	45	+++	−	Little in some	
E. invadens	P.Z.	Bacteria-free	10	+++	−	Little in some	

NOTE: −   No amoebae or cysts seen after microscopic examination.
        +   1–20 amoebae or cysts seen per microscope field at low power.
       ++   20–50 amoebae or cysts seen per microscope field at low power.
     +++   50–100 amoebae or cysts seen per microscope field at low power.
   ++++   Over 100 amoebae or cysts seen per microscope field at low power.

The reason why the trophozoites in some of the mixed cultures failed to encyst was not clear. Presumably it was because of accidental contamination of these cultures with an unfavorable species of bacterium, which became dominant. In any case it was apparent that in those cultures where mass encystation did not occur, the trophozoites failed to accumulate glycogen.

Because mass encystation in cultures could be correlated with a heavy growth of trophozoites, it seemed probable that the amoebae themselves must contribute a factor favoring encystation. This intrinsic factor might be an accumulation of metabolic wastes in sufficiently high concentrations, or some material produced as a result of overcrowding. This view has been previously expressed by Everitt (5).

The intrinsic factor was presumed to be present to some extent in bacteria-free cultures of *E. invadens* which contained a relatively large number of trophozoites. Because it was observed that bacteria-free trophozoites did not accumulate much glycogen, it seemed likely that if a sterile polysaccharide or mucopolysaccharide were added to well-established bacteria-free cultures, then some encystation might be expected to occur.

The results of seven experiments involving 71 bacteria-free cultures of the three strains of *E. invadens* ranging in age from fourteen to seventy days, to which mucin suspensions in final concentrations of 0.1–1%, glycogen solution in final concentrations of 0.1–1%, rice starch, and suspensions of autoclaved bacterial cells were added either individually or in combinations, are summarized in Table II.

TABLE II

ENCYSTATION OF BACTERIA-FREE *E. invadens* FOLLOWING THE
ADDITION OF VARIOUS SUBSTANCES TO THE CULTURES

Addition to cultures	Total no. of cultures	No. of cultures in which encystation occurred	% of cultures in which encystation occurred
Mucin suspension	36	28	78
Glycogen solution	8	0	0
Rice starch	5	0	0
Autoclaved bacteria	9	7	78
Mucin+starch	4	1	25
Mucin+glycogen	1	0	0
Mucin+autoclaved bacteria	5	5	100
Glycogen+autoclaved bacteria	3	1	33
Controls (no additions)	40	0	0

In another series consisting of 10 bacteria-free cultures of each of the three strains of *E. invadens*, mucin suspension to the final concentration of 0.1% was added to five cultures and autoclaved bacteria to the other five of each strain, at the time of initiation of the cultures. When the cultures were examined at the end of 20 days, no cysts were seen in any of them, although the multiplication of the trophozoites was evident.

It was concluded that the reason why encystation did not take place in cultures which received mucin or autoclaved bacteria at the time of initiation, was because the amoebae fed on mucin particles and on the dead bacterial cells during the period of active multiplication. Thus, the amount of mucin and of bacterial suspensions was depleted before there was a sufficiently high concentration of the amoebal metabolic product. This showed that two factors, an ingestible polysaccharide (which was contained in the mucin particles and in the bacterial cells), and the product of amoebic metabolism, had to be present simultaneously in order for the processes leading to encystation to take place.

In order to demonstrate the effect of bacteria on the encystation of bacteria-free *E. invadens* in the presence and absence of ingestible polysaccharides, a

technique of double culture was devised. This approach rested on the supposition that the products of bacterial metabolism, responsible for the encystation of amoebae, would be dialyzable.

A total of 24 double cultures was set up. In the first nine, bacteria-free *E. invadens* of the I.P.-2 strain was cultured in the inner cellophane tubes. In four of these nine double cultures the outer tubes contained mixed cultures of the same strain of *E. invadens*, in three others only the bacteria associated with the amoebae in the mixed cultures, and in the remaining two, only sterile Balamuth's medium. When the bacteria-free components of the double cultures were examined 7 to 10 days later, some encystation occurred in all cases, except in the two where the outer tubes contained nothing but sterile medium. Eight control cultures, set up in the usual bacteria-free medium at the same time as the double cultures, and examined on the same days, had no cysts.

The experiments involving the remaining 15 double cultures (Nos. 10–24) are summarized in Table III.

The double-culture experiments demonstrated that a dialyzable factor was produced by the bacteria in mixed cultures. This factor had some effect on the encystation of bacteria-free trophozoites of *E. invadens*. There was no significant difference in encystation when the outer cultures contained bacteria alone or *E. invadens* with bacteria. The percentage of cysts relative to the total population increased with the period of incubation.

TABLE III

ENCYSTATION OF BACTERIA-FREE *E. invadens* BY MEANS OF THE DOUBLE-CULTURE TECHNIQUE, THE OUTER TUBES CONTAINING MIXED CULTURES AND THE INNER TUBES CONTAINING BACTERIA-FREE CULTURES OF I.P.-1 STRAIN OF *E. invadens*

Double culture No.	Additions to inner tubes	Examined after, days	Encystation in bacteria-free cultures in % (cysts/total population)
10	Nil	6	Less than 1
11	Nil	11	13
12	Starch	8	3
13	Starch	8	4
14	Mucin to 0.1%	8	7
15	Mucin to 0.1%	6	9
16	Mucin to 0.1%	11	31
17	Autoclaved bacteria	8	3
18	Autoclaved bacteria	6	1
19	Autoclaved bacteria	11	7
20	Starch+autoclaved bacteria	8	15
21	Starch+autoclaved bacteria	8	8
22	0.1% mucin+autoclaved bacteria	8	10
23	0.1% mucin+autoclaved bacteria	6	7
24	0.1% mucin+autoclaved bacteria	11	22
Ten control bacteria-free cultures of *E. invadens*, I.P.-1 strain		6, 8, or 11	0

Discussion

The results of these experiments have indicated that the following factors were necessary for the encystation of *E. invadens* in vitro:

1. presence of particulate, ingestible food (polysaccharide or mucopolysaccharide) which could be converted to glycogen;

2. presence of a factor or factors elaborated by living bacteria;

3. presence of an intrinsic factor or factors elaborated by the amoebic trophozoites prior to mass encystation. The concentration of this factor in the culture is proportional to the population density of the amoebae and probably is a result of excessive growth and overcrowding.

When all the three factors were present (as in the mixed cultures), mass encystation occurred. At least two of the factors were necessary to cause any encystation. Mass encystation in cultures must be regarded as a result of very favorable environmental conditions, because it follows a period of active growth and multiplication of the trophozoites. Because it was seen that the intrinsic factor played a part in encystation, it is possible to suppose that it had the function of activating the chain of events leading to encystation when its concentration in the culture reached a certain minimum level. In other words, the conditions favorable to the multiplication of trophozoites became unfavorable at this minimum concentration and the encystation process was initiated. Factors 1 and 2, listed above, were necessary for the growth and multiplication of the trophozoites; factor 3 became effective in bringing about mass encystation, only if factors 1 and 2 have been utilized.

The conclusions derived from these observations suggested the formulation of the following hypothesis. The trophozoites, being phagotrophic by nature (9), ingested starch granules or particles of mucin. The ingested particles, surrounded by a thin film of the fluid from the outside, were transported into the food vacuoles of the amoebae. The film of fluid must have contained in solution certain products of bacterial metabolism from the culture. It is assumed that the products of bacterial metabolism (perhaps in conjunction with some secretions produced by the amoebae themselves) acted on the ingested food particles within the food vacuoles and broke them down to simpler molecules. The synthesis of glycogen could then proceed. When most of the amoebae in a culture had accumulated enough glycogen, and if by that time the intrinsic factor was present in the culture in a certain minimum concentration, then mass encystation took place.

The question whether parasitic amoebae produce amylolytic enzymes independent of the associated bacteria has not been answered conclusively. It was reported that *E. terrapinae* produced amyloclastic enzymes independent of, but conditioned by the associated bacteria (10), that *E. histolytica* possessed amylolytic enzymes even in the absence of bacteria (3), and that it produced a gelatinase and/or a protease which liberated starch grains from particles of ground rice, and that rice proteolysis might be adaptive (15).

In monobacterial cultures, with a streptobacillus as the only associate, it was reported that *E. histolytica* depends on the bacteria for its supply of glucosamine because it is unable to utilize glucose as such (8).

The importance of particles in the cultures of *E. histolytica* has also been appreciated by other writers (1). According to them, "whatever stimulation resulted from addition of materials in solution occurred only in the presence of particles," which confirms the theory that amoebae may ingest liquids only when they are carried into the vacuoles together with solid particles.

In another study on *E. histolytica*, *E. invadens*, and *E. terrapinae* (6) it was concluded that if these organisms possess amylolytic enzymes which are intracellular and not secreted into the medium, any soluble starch would not be available since it could not be ingested, and consequently the polysaccharide food must be solid.

In these experiments it was seen that rice starch was ingested, but not digested by *E. invadens* in the absence of bacteria, and consequently little or no glycogen was accumulated.

It has been noted by the writer and by Dr. N. R. Stoll (personal communication) that *E. invadens* seems to have a special predilection for mucin in cultures. The same is true for the situation in vivo, as histological sections of infected snake intestines show concentrations of the parasites feeding on mucus in spaces between the villi, the mucus-secreting goblet cells hypertrophied and sometimes being actually invaded by the amoebae.

It is suggested here that a state of commensalism between *E. invadens* and the turtles can exist because the amoebae are able to derive polysaccharide food of plant origin in the intestinal lumen of turtles, which are at least partially herbivorous. Thus the amoebae are able to accumulate glycogen, encyst, and complete their life cycle without damaging the host.

In the intestinal lumen of snakes, which are all exclusively carnivorous, such a source of food for the amoebae is not available, and they resort to feeding on the mucous secretion of the intestinal epithelium, eventually denuding the intestinal mucosa of the protective mucus envelope and gaining entry into the deeper tissues. As more and more of the mucosa becomes exposed, it becomes subject to the action of bacteria and to autodigestion. The extensive pathological changes in the intestines of snakes infected with *E. invadens* is most probably not due to the amoebae alone. They are initiated by the amoebae, but the main contributing factors are secondary in nature. It appears that the reason why cysts are never formed in the extraintestinal phase of snake amoebiasis, is because the three factors necessary for encystation are not available in the tissues, or may be available, but in too low concentrations.

Biologically speaking, the presence of trophozoites of parasitic amoebae in tissues of their hosts is a dead end. They are neither able to encyst, nor to reach the exterior. This cannot be a manifestation of the normal life cycle of the species. Tissue invasion by *E. invadens*, and by analogy *E. histolytica*, must be an accident due to their presence in an abnormal host.

The writer's attempts to verify a statement by Neal (14) that *E. invadens* in the tissues of snakes encysts upon the death of the host have not succeeded. Infected livers, kidneys, and other tissues were allowed to decompose dry and in water under aerobic and anaerobic conditions, but the amoebae disappeared and no cysts were ever found. It appears, therefore, that the transmission of *E. invadens* from snake to snake in nature is very unlikely.

The host–parasite relations between *E. invadens* and turtles follow a basic parasitological postulate that the longer the association between the parasite and the host, the more commensal the parasitism becomes. Phylogenetically, turtles are much more ancient than snakes. Being at least partially vegetarian they could have easily acquired a free-living amoeba, which with the course of time had established itself as an obligate parasite. It is interesting to note that among a number of species of *Entamoeba* parasitic in reptiles, only *E. invadens* had been observed in snakes. All the other species are harmless commensals in turtles. Amoebiasis has been observed or induced in lizards and the available information corroborates the hypothesis of host–parasite relations in reptile amoebiasis: carnivorous lizards are subject to pathological changes, disease, and death, while in the herbivorous ones the infection remains asymptomatic.

The tissue-invading tendency of *E. invadens* and *E. histolytica* cannot be attributed directly to the nutritional and hostal environments. These species are able to produce histolytic or cytolytic enzymes. The question whether the production of these enzymes is conditioned by the presence of amoebae only in abnormal hosts is still to be answered.

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REDESCRIPTION OF CHORIOPTES TEXANUS, A PARASITIC MITE FROM THE EARS OF REINDEER IN THE CANADIAN ARCTIC[1]

Gordon K. Sweatman[2]

Abstract

Chorioptes texanus, which was first described by Hirst and collected from the bodies of domestic goats in Texas, is redescribed from the ears of semidomesticated reindeer in the Canadian Arctic.

Introduction

At the present time, the genus *Chorioptes* contains two species of mange mites. There is *C. bovis* (Héring, 1845) Gervais and van Beneden, 1859 from the feet, legs, and torso of the domestic cow, goat, horse, sheep, llama (*Lama huanacus glama*), guanaco (*Lama huanacus*), and Barbary sheep (*Ammotragus lervia*). Sweatman (3, 4, 5) reported on the biology of this mite, and demonstrated that *C. bovis* has a broad potential host range among various domestic and wild ungulates. *C. texanus* Hirst, 1924 (1) is the second species in the genus and was collected on one occasion from the bodies of domestic goats in Texas. In addition to these, Lavoipierre (2) is describing a new species of *Chorioptes* that is morphologically distinct from both *C. bovis* and *C. texanus* which was collected from the ears of a red-flanked duiker (*Cephalophus rufilatus*) in the British Cameroons.

Hirst's description of *C. texanus* was short. He pointed out that this parasite could be differentiated from *C. bovis* in the adult male stage by (*a*) the presence of narrower blade-like setae on the opisthosomal lobes, and by (*b*) a short, fine seta at the outer corner of each opisthosomal lobe which contrasted with the long, thick seta in the same position on *C. bovis*. The current writer found mites in the ears of semidomesticated reindeer (*Rangifer tarandi*) at Reindeer Depot, N.W.T., that conform to Hirst's description and which also resemble Oudeman's photograph of a male *C. texanus* reproduced somewhat unsatisfactorily by Vitzthum (6). The original mite, of which there appears to be only a single specimen in existence, is type material, and because of this, could not be released from the British Museum of Natural History. The present redescription is therefore a development from the above-mentioned notes of Hirst and photograph of Oudemans.

Redescription of *C. texanus*

The larva, male and female protonymphs, male deutonymph, pubescent female, and ovigerous female of *C. texanus* collected from the reindeer are morphologically identical with the same stages of *C. bovis*. An account of

these may be found elsewhere (4).   Only the posterior halves of the adult males can be differentiated.   Besides the criteria noted by Hirst, the present author will point out additional diagnostic features, both absolute and relative. Table I summarizes the data on the comparative lengths of the setae mentioned herein.   Each opisthosomal lobe on the adult male of *C. texanus* has a primary lobe with an outer, smaller secondary lobe, whereas *C. bovis* has a single pair of undivided lobes (Figs. 1 and 2).   The secondary lobe of *C. texanus* gives rise to a short, fine seta (noted by Hirst), while a long, thick seta in *C. bovis* arises from the outer corner of the single opisthosomal lobe on the same plane as the prominent spatulate setae.   In both species, the spatulate setae arise from the posteromedial corner of the opisthosomal lobe, but those of *C. texanus* are almost twice as long as those of *C. bovis*. The outer spatulate seta of *C. bovis* has a conspicuous hook towards the proximal end.   This hook is absent on *C. texanus*.   Arising just anterior to the innermost spatulate seta is a short seta in *C. texanus*, whereas its counterpart in *C. bovis* is four times longer and arises more anteriorly.   Lateral to the opisthosomal lobes, the dorsum of both species ends posteriorly in a small, but distinct, point.   Arising near or from the point is a short seta in *C. texanus*, but the corresponding seta is three times as long on *C. bovis*.   In *C. bovis*, tarsus III supports one ventral and two dorsal setae as well as a caruncle, a hook with two teeth, and a smaller ventral hook.   The single ventral seta is four times longer in *C. bovis* than it is in *C. texanus*.   These data show that there are more than a half dozen reliable criteria for differentiating *C. texanus** from *C. bovis*.

TABLE I

COMPARATIVE LENGTHS OF SETAE ON ADULT MALES

(The numbers below correspond to the setae so-marked in Figs. 1 and 2)

Setal No.	Setal length,† μ				
	1	2	3	4	5
C. texanus					
Range	66–98	193–235	27–39	23–27	14–29
Mean	80	216	31	25	18
Standard deviation of individuals	7.5	10.2	2.9	1.4	3.3
C. bovis					
Range	267–375	103–126	103–135	61–95	71–111
Mean	315	115	117	77	84
Standard deviation of individuals	30.5	6.5	9.3	10.1	11.4

†In all cases, 20 measurements were made of each seta.
*Specimens collected in the present study have been deposited with Dr. G. Owen Evans in the British Museum of Natural History.
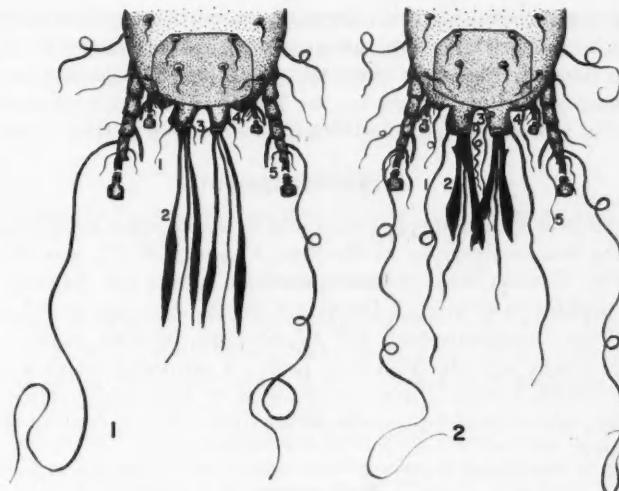
FIGS. 1 and 2. Hysterosomal region of adult male of *Chorioptes texanus* and of adult male of *Chorioptes bovis*. Comparative measurements for the numbered setae are presented in Table I.

Both known hosts of *C. texanus*, the goat and reindeer, are introductions into America, the latter having come from Lapland in 1929 to Canada via a route through Alaska. During the drive across Alaska, and subsequently at Reindeer Depot, N.W.T., there has been occasional contact and some crossbreeding with the indigenous barren-ground caribou (*Rangifer arcticus*). No caribou were available for study, but the ears of four other cervids native to other parts of America were examined for the presence of ear mites. These included two white-tailed deer (*Odocoileus virginianus*) from Quebec; seven moose (*Alces alces*) and 40 wapiti (*Cervus canadensis*) from Elk Island Park, Alberta; and five mule deer (*O. hemionus*) from New Mexico. From this same state one pronghorn (*Antilocapra americana*) was examined also. Among domestic animals, three Quebec herds of Holstein–Friesian, Ayrshire, and Shorthorn cattle and two herds of goats with natural infestations of *C. bovis* on their bodies were examined for ear mites. The ears of two other goats from New Mexico were examined also. None of the above animals was infested.

The incidence of *C. texanus* was not noted, but appeared high in the 517 reindeer slaughtered. The mites were virtually non-pathogenic although a few reindeer contained some excess cerumen. A few body areas on the reindeer were examined, some following digestion of the skin in potassium hydroxide, and no mites were observed. Hirst (1) received information which indicated that the mites on the bodies of the goats were somewhat pathogenic. Their ears were not examined presumably, but it is possible that these were infested also. Other mites in the family Psoroptidae, for

example *Psoroptes cuniculi*, are essentially ear-infesting, but on occasion parasitize the body where they are generally more pathogenic than when confined to the ears.   It seems likely that *C. texanus* is essentially an auricular mite that sometimes occurs on the body.   The above data show that *C. texanus* is morphologically and biologically distinct from *C. bovis*.

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ON SOME HELMINTH PARASITES COLLECTED FROM THE MUSK OX (OVIBOS MOSCHATUS) IN THE THELON GAME SANCTUARY, NORTHWEST TERRITORIES[1]

HAROLD C. GIBBS AND JOHN S. TENER

Abstract

Three species of nematodes and three species of cestodes are reported from *Ovibos moschatus* taken in the Thelon Game Sanctuary, N.W.T.

Introduction

During the summer of 1957 in the course of the Canadian Wildlife Service study on musk oxen, the authors had the opportunity of collecting some helminth parasites from three adult males of this species taken under permit in the Thelon Game Sanctuary, N.W.T.

Probably the first report of parasites from musk ox was that of Fielden (6), who in 1877 reported the finding of two species of helminths from animals taken in east Greenland. These were *Taenia* sp. and *Filaria* sp. Later, Jensen (9) in 1904 found "a tapeworm in the bladder phase" in the liver of an animal taken at Hurry Inlet, Greenland. On the North American continent, Dikmans (3) in 1939 listed four species of helminths from the musk ox, three nematodes and one cestode, but made no reference to the locality from which the animals were obtained. More recently Tener (17) reported finding a species of cestode in an animal taken on Ellesmere Island, N.W.T.

In view of the scarcity of literature on the parasites of this host species it was felt that the authors' findings should be published.

List of Parasites

NEMATODA

METASTRONGYLIDAE LEIPER, 1908

Dictyocaulus viviparus (Bloch, 1782)

This parasite was found in the bronchioles of the lungs of all three animals. There was a fairly heavy infestation resulting in a nodular fibrosis at the margins of the diaphragmatic lobes. Portions of the lung surface showed raised emphysematous areas caused by air trapped when the bronchioles were blocked by worms and debris.

Microscopic examination revealed changes characteristic of verminous bronchitis.

This is the first record of the parasite from this host in the wild state, although it has been reported by Durrell and Bolton (5) from a calf in Vermont.

TRICHOSTRONGYLIDAE LEIPER, 1912: TRICHOSTRONGYLINAE LEIPER, 1909

Nematodirella longispiculata (Romanovitch, 1915)

This nematode was found in the duodenum and anterior jejunum of one animal. It is characterized by the presence of very long spicules in the male and non-development of the anterior horn of the uterus in the female. When fresh these worms present a reddish appearance and in the female the white coils of the uterus are easily seen.

There was some evidence of parasitic enteritis in the duodenum of the animal infested. There were petechial haemorrhages, some denuding of the mucosa, and a quantity of mucus in the lumen. In addition reddish plaque-like areas were observed on the walls of the duodenum in the area where these worms were found. Microscopic examination of these areas revealed the presence of a granulomatous-type lesion of the submucosa. There was no evidence of acid-fast bacilli and it is debatable whether this lesion was the result of parasitism.

This is the first record of the parasite from this host. It had previously been reported by Romanovitch (12) from reindeer, and from moose and prong-horn antelope by Dikmans (3).

Ostertagia circumcincta (Stadelman, 1894)

This parasite was found at the pyloric end of the abomasum in all three animals. In one animal the pylorus was thickly covered by numerous worms but they appeared to cause little damage. In the other animals only a few individuals were seen.

This species was previously reported from the musk ox by Dikmans (3).

CESTODA

ANOPLOCEPHALIDAE CHOLODKOWSKY, 1902

Moniezia expansa (Rudolphi, 1805)

A number of these worms were obtained from the ileum of all three animals. There is still much conflicting evidence on the pathological significance of these worms. The animals examined did not appear to be affected.

Tener (17) previously reported finding an unidentified species of this genus in a 2-year-old bull he examined on Ellesmere Island, N.W.T.

TAENIIDAE HALDEMAN, 1851

Cysticercus tenuicollis Rudolphi, 1810

This larval form of *Taenia hydatigena* Pallas, 1776 was found in a number of locations on all three animals. The cysts, both viable and calcified, were found on the surface of the liver as well as deep in the liver tissue. Some were also found in the greater omentum and on the peritoneum. Choquette *et al.* (2) have drawn attention to the fact that development of the cysts deep in

the liver tissue is different from that seen in domestic sheep where, although cysts may be seen occasionally on the surface, they are never found deep in the tissue.

This parasite, which is common in northern herbivores, was previously described from musk oxen by Dikmans (3).

Echinococcus granulosus (Batsch, 1786)

A cyst of this parasite which appeared to be multilocular and sterile on gross examination was found in the lungs of one animal.   Microscopic examination confirmed the belief that it was sterile as, though a laminated membrane was visible, there was no evidence of scolices.   This is the first record of the parasite in this host.

Discussion

The helminth fauna of this species of host is seen to be typical of that found in northern ruminants.   These parasitic species apparently exhibit great resistance to the effects of low environmental temperatures as shown by the work of Griffiths (8), Furman (7), Dinaburg (4), Schiller (14), and others.   All of the species reported in this survey are found in a number of different species of ruminant host and certainly occur in all of the ruminant hosts found in the Northwest Territories.   It, therefore, appears that resistance to low environmental temperatures coupled with a versatility in choice of host have enabled these parasites to exploit their ecological niche to the fullest extent.

In 1957 Durrell and Bolton (5) claimed that a female musk ox calf which had died of haemonchosis on a farm in Vermont, U.S.A., probably became infected in its native environment, which was the Thelon Game Sanctuary.   It is felt that this assumption is erroneous.   It has been shown by Griffiths (8), Shorb (15), Kates (10), Sarles (13), and recently Belle (1) that the free-living stages of *Haemonchus contortus* are not very resistant to temperatures below freezing.   It is unlikely, therefore, that this parasite could survive the severe winter conditions present in the Thelon Game Sanctuary.   Furthermore, this parasite has never been reported before from northern ruminants.   The musk ox in question had been put on to pasture in the spring with domestic cattle and goats which "were said to have been negative for parasites", and had been treated with phenothiazine as a further precautionary measure.   The domestic cattle were later found infected with stomach worms which the authors considered to have been contracted from the musk ox.   However, probably the reverse was true as the strain of parasite found was the bovine strain, *H. placei*.   It appears from serological findings by Moody (11) that the musk ox is more closely related to goats than to cattle.   It is a universally held precept that the establishment of a parasite in a new or unusual host usually elicits a severe pathological response.   What probably happened, therefore, was that the musk ox contracted its fatal infection from the cattle when the two species were put together on pasture in the spring.

Check list of helminth parasites recorded from musk oxen collected in their natural habitat

Parasite	Location in host	Locality	Author
Dictyocaulus viviparus	Lungs	Thelon	Present study
Nematodirella longispiculata	Duodenum, jejunum	Thelon	Present study
Ostertagia circumcincta	Pylorus of abomasum	N. America, Thelon	Dikmans (3), present study
O. mossi	Abomasum	N. America	Dikmans (3)
O. occidentalis	Abomasum	N. America	Dikmans (3)
Filaria sp.		E. Greenland	Fielden (6)
Moniezia sp.	Ileum	Ellesmere Island	Tener (17)
M. expansa	Ileum	Thelon	Present study
Taenia sp.		E. Greenland	Fielden (6)
Bladder phase of tapeworm	Liver	Hurry Inlet	Jensen (9)
Cysticercus tenuicollis	Liver, omentum, peritoneum	Thelon, N. America	Present study, Dikmans (3)
Echinococcus granulosus	Lungs	Thelon	Present study

Acknowledgment

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TRANSMISSION OF LEUCOCYTOZOON BONASAE CLARKE TO RUFFED GROUSE (BONASA UMBELLUS L.) BY THE BLACK FLIES SIMULIUM LATIPES MG. AND SIMULIUM AUREUM FRIES[1]

A. M. FALLIS AND G. F. BENNETT ·

Abstract

Specimens of *Simulium aureum, S. latipes, S. venustum,* and *S. rugglesi* feed on ruffed grouse although relatively few engorged specimens of the two latter species were obtained. *S. aureum* and *S. latipes* are suitable hosts for *Leucocytozoon bonasae* as shown by infections produced in grouse following injections of sporozoites removed from the salivary glands of these flies. The parasitaemia in grouse infected naturally and experimentally was relatively low and no gross signs of disease were noted in any of the infected birds.

Introduction

The common occurrence of *Leucocytozoon bonasae* in ruffed grouse in Ontario (3, 8) and elsewhere (6) has attracted attention for some time, although the vector of the parasite and consequently its life history has remained unknown. This applies also to *Leucocytozoon* in other species of grouse in other places where an equal, or even higher, incidence of the parasite has been noted (1, 4, 12). Recent observations on the species of simuliids that feed on ducks (10) and preliminary observations of those feeding on other kinds of birds indicated differences in the feeding habits of the various species of Simuliidae. During the course of this work there was an opportunity to collect simuliids that had fed on ruffed grouse infected with *L. bonasae.* It will be shown that *Simulium latipes* and *S. aureum* of the subgenus *Eusimulium* are suitable intermediate hosts for this parasite.

When Clarke (3) described *L. bonasae* it was suspected to be a cause of the periodic decline reported for grouse populations. This seemed reasonable as Wickware (15) and O'Roke (13) had reported that *L. simondi* caused a high mortality in ducks. Observations of natural and experimental infections with *L. bonasae* have been made therefore to determine whether it is equally pathogenic. Observations and experiments relating to this study were made at the Wildlife Research Station of the Department of Lands and Forests, Lake Sasajewan, Algonquin Park, Ontario.

Materials and Methods

Grouse chicks were obtained from wild broods a few days after hatching when their capture was feasible. Following capture the grouse chicks were kept indoors in a cage 24 in. ×18 in. ×9 in., one half of which was made of wood and the remainder covered with screen (20 mesh to the inch). Heat, which was most essential for survival of the chicks, was provided by a 60-w. electric light

bulb attached to the solid end of the cage.   The bottom of the cage in which the grouse were kept indoors was covered with a layer of soil, moss, and plants from the forest floor.   This was renewed every 3 to 4 days.   Clean water was supplied twice daily in shallow containers partially filled with stones.   In this way only a small surface of water was exposed and the small chicks could not easily become wetted.   Reasonable success in rearing grouse chicks has been obtained with the following diet.   Chicks less than one week old were fed as many insects as they would eat.   These were obtained by sweeping with a net in a meadow of short grass and weeds.   The grouse were supplied at the same time with finely chopped boiled eggs, crushed fox and dog pellets, chicken starter mash, cracked buckwheat, and millet seed.   Sprouting buckwheat and broken leaves of various plants were provided also from time to time and were eaten readily by the grouse.   Initially the chicks fed chiefly on insects.   As they grew older, larger amounts of the other foods were eaten and fewer insects were provided.   When they were about two months old they were fed chiefly on chicken mash, fox pellets, and buckwheat and small amounts of green stuff were provided periodically.   The food trays were cleaned daily, although food was left with the chicks at all times.   Adult grouse were fed throughout the winter on buckwheat and occasionally small amounts of lettuce.

Most of the grouse chicks were held indoors for at least two weeks after capture although when the weather was suitable a few were placed outdoors for short periods before and after this time so they might become infected naturally.   Blood smears were made from each of them for at least three weeks after capture to determine whether they were infected prior to capture.

As an indication of transmission of *Leucocytozoon* during the summer, blood smears were made also from young grouse following their exposure to natural and experimental infections and from others that were shot.   An estimate of the parasitaemia in the grouse on successive days was obtained from counts of the number of parasites seen in the blood smears during 10 minutes' observation at a magnification of 300 diameters.

Results

It was assumed from our knowledge of the vectors of other species of *Leucocytozoon* that some simuliid would be a suitable host for *L. bonasae*.   In fact a few years ago the early development in *Simulium venustum* was noted, although the observation was never published.   This species was suspected of being a vector.   However, as will be shown below, we have no proof of this.   Data on the life cycle were obtained by collecting specimens of simuliids that fed on infected grouse, dissecting and examining these at intervals thereafter, and, when sporozoites were observed in the salivary glands, injecting subcutaneously suspensions of these sporozoites in physiological saline solution into "clean" grouse.

The following simuliids fed on grouse: *S. aureum*, *S. latipes*, *S. venustum*, and an occasional specimen of *S. rugglesi*.   The two former were taken more often than the others.   *L. bonasae* will develop to the oökinete stage (Fig. 1)

PLATE I
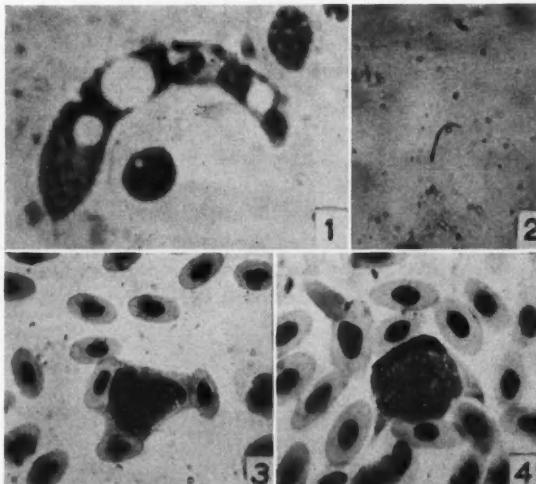
FIG. 1. Oökinete of *L. bonasae* from stomach of black fly 8 hours after ingestion of gametocytes.

FIG. 2. Sporozoite, presumably that of *L. bonasae*, from salivary gland of *Simulium aureum*. Similar sporozoites from this and other flies produced infection when injected into grouse.

FIG. 3. Female gametocyte of *L. bonasae* in a host cell that lacks the attenuated ends.

FIG. 4. Female gametocyte of *L. bonasae* in a host cell having attenuated ends.
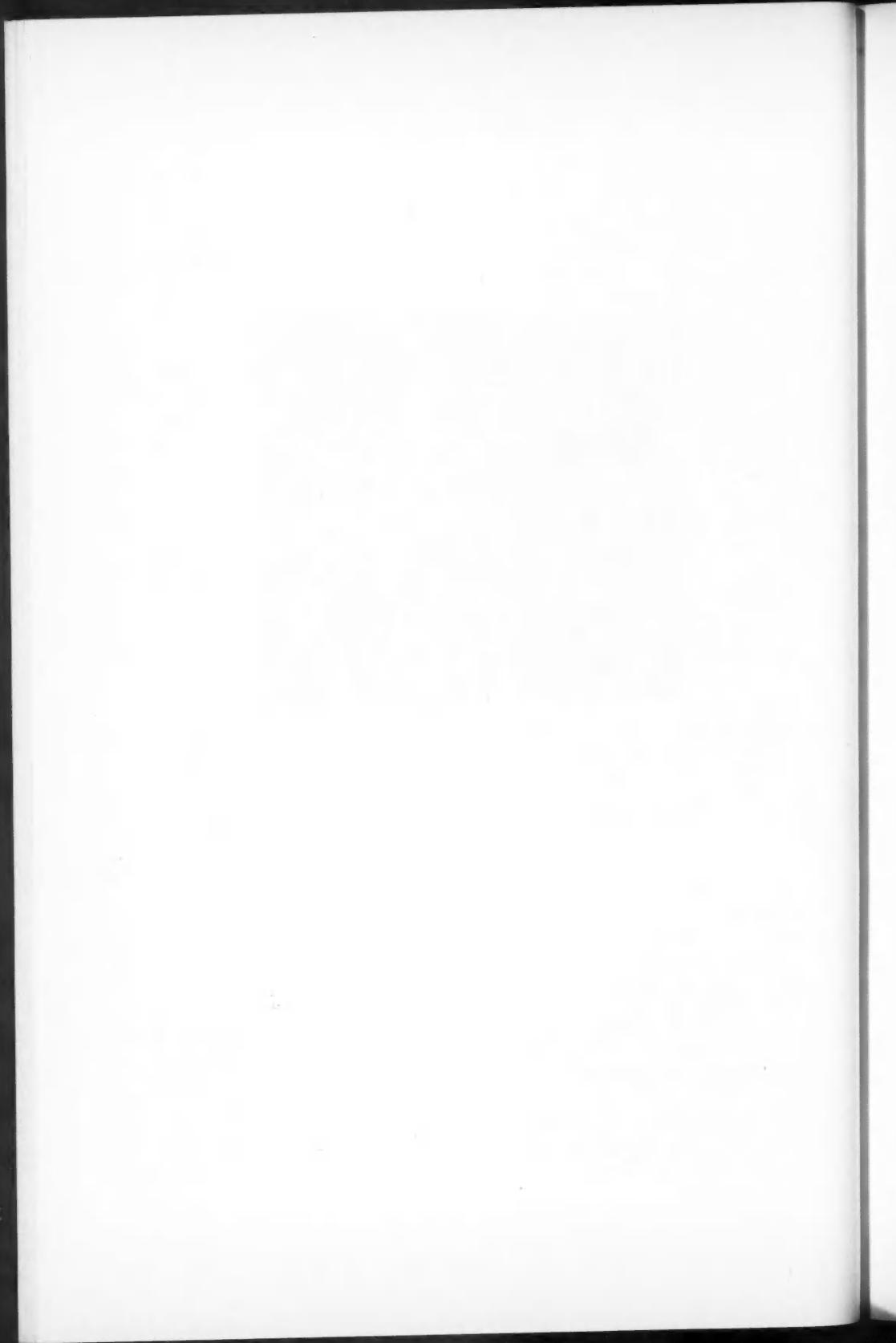
in all of them. Sporozoites (Fig. 2), known to be infective to grouse, have been found in the salivary glands in *aureum* and *latipes* but not in *venustum*. Infections developed in three young grouse that were inoculated with saline suspension of sporozoites from two and three specimens respectively of *latipes* and *aureum* but not in grouse inoculated with saline suspensions of specimens of *venustum* that had fed 4 to 10 days previously on infected grouse (Table I). Failure to produce infection with specimens of *venustum* that ingested gametocytes only 3–5 days before could result from lack of time for sporogony. However, since infections did not develop in birds that were inoculated with flies that ingested gametocytes 6 to 10 days previously, it seems unlikely that *venustum* is a suitable host.

The sporozoites inoculated into grouse No. 18 were from black flies that had fed only 36 hours before on an infected grouse. Most probably these sporozoites developed from gametocytes that were ingested several days previously rather than from those taken 36 hours before. None of the flies used for these injections were reared in captivity, consequently their history is unknown prior to our collection of them from an infected grouse. A complete knowledge of the sporogony in the fly must await the study of a larger series

TABLE I

SUMMARY OF EXPERIMENTAL TRANSMISSION OF *L. bonasae* TO RUFFED GROUSE

Grouse				Species of fly and time since it last fed on infected grouse		Remarks
No.	Approx. age, weeks	Date injected	Date parasites seen			
18	4	12/7	25/7	S. latipes	36 hours	Sporozoites from salivary glands of 2 flies
12	4	16/7	29/7	S. aureum	8 days	Sporozoites from salivary glands of 3 flies
15	4	17/7	29/7	S. aureum	7 days	Injected one third of a suspension of sporozoites from 8 flies
284	3	6/7	–	S. venustum	8 days	Suspension of 2 flies
284	4	10/7	–	S. venustum	8 days	Suspension of 3 flies
284	5	21/7	–	S. venustum	6 days	Suspension of 3 flies
285	4	10/7	–	S. venustum	10 days	Suspension of 1 fly
285	6	26/7	–	S. venustum	5 days	Suspension of 7 flies
284	6	26/7	–	S. venustum	4 days	Suspension of 8 flies
284	7	1/8	–	S. venustum	3 days	Suspension of 11 flies
285	7	1/8	–	S. venustum	4 days	Suspension of 4 flies
285	10	2/9	–	S. venustum	4 days	Suspension of 1 fly

of flies on successive days after feeding on infected grouse and the examination
of an adequate number of controls.   The specificity of *L. bonasae* is indicated
by the fact that grouse No. 15 was infected as a result of the inoculation of
sporozoites obtained from specimens of *aureum*, but infection was not detected
in a duckling and a white crown sparrow that were inoculated with suspensions
of sporozoites from the same flies.

Sporozoites from the salivary glands of specimens of *aureum* and *latipes*
similar to those that were injected into the grouse measure $1-2\,\mu \times 7-12\,\mu$ in
dried preparations fixed with absolute alcohol and stained with Giemsa.   Some
are shorter and thicker than others, but all are characteristically more pointed
at one end than the other and resemble those described for *L. simondi*.   The
cytoplasm is vacuolated and the nucleus, although frequently nearer the pointed
end, occurs near the center in some sporozoites and slightly toward the blunt
end in others.   Possibly the sporozoites of more than one species of *Leuco-
cytozoon* were present in the flies that were dissected, for several gorged speci-
mens of *latipes* have been taken from a grackle, blue jay, raven, great blue heron,
white-throated sparrow, and purple finch as well as from grouse, and each of
these birds harbors species of *Leucocytozoon*.   *S. aureum* was taken also from
each of these birds except the purple finch and white-throated sparrow.   Ex-
tensive studies of sporozoites of known identity will be necessary to determine
whether specific differences exist among them.

Parasites were detected in the peripheral blood 12 and 13 days respectively
after sporozoites were injected into the grouse.   Most probably a few small
parasites were present at least two days earlier, assuming that the rate of
growth is similar to that of *L. simondi*, and an even shorter period might be
expected in heavy infections.   None of the parasitaemias observed in grouse
infected either naturally or experimentally are as intense as many of those
seen in ducks infected with *L. simondi*.   The highest parasitaemia (*ca*. one
parasite per 300 red blood cells) observed thus far (Fig. 5) resembles one of the
lowest seen in ducks (9).   Forty-three per cent of 300 of these parasites were
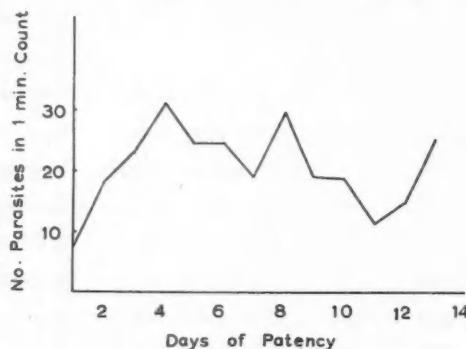
FIG. 5.   Parasitaemia observed in a grouse infected with *L. bonasae*.   The graph was
constructed from the count of the number of parasites seen in stained films in 10 minutes
at a magnification of 300×.

microgametocytes and the balance were macrogametocytes—a ratio similar
to that known for other species of *Leucocytozoon*. The scarcity of small
parasites in the peripheral blood may be related to the low-grade infections
that occurred.

Too few small parasites have been seen to say, with certainty, which of the
blood cells are invaded most often by the parasite. Some have been noted
in erythroblasts. Most of the parasites seen at the beginning were irregularly
round and in host cells of similar shape (Fig. 3). Others were in cells that
had a short attenuated portion on two sides while other host cells had the
attenuated ends that are described as typical (Fig. 4). The appearance of the
host cell, and the shape of the parasite within, need further study for actually
the round parasites in round cells are mature as shown by their capacity to
exflagellate in vitro. Possibly some parasites that are in cells with pronounced
attenuated ends are postmature and incapable of further development as
appears to be the case for some of the specimens of *L. simondi* in attenuated
cells (9).

Exflagellation of microgametocytes has been noted within one minute after
blood containing them has been withdrawn into saline on a slide and covered
with a cover glass. Presumably it can occur as rapidly in the stomach of a
black fly. The rate varies undoubtedly from one specimen to another.
Oökinete formation will take place on the slide within four hours after the
blood has been drawn into the saline. Twenty oökinetes in an air-dried
smear of the stomach content of a black fly stained with Giemsa measured
$2-4\mu \times 24-31\mu$ (av. $3\times27\mu$) (Fig. 1).

An indication of the time of year that young grouse became infected with
L. bonasae was obtained by examining the blood of (*i*) those killed in the field
at different times throughout the summer and (*ii*) those kept in captivity
throughout the summer. Table II summarizes these observations.

Large parasites were present in some of the grouse chicks at the end of
June and beginning of July (Nos. 195, 254, 289, 307, 308, 309, 310). Obviously,
with a prepatent period of 12 days, infected flies must have been in the area
about mid-June. These flies must have acquired their infections from other
birds several days prior to this time. Parasites were not detected in other
chicks until early September (Nos. 348, 361). Consequently, transmission may
occur over an extended period. *S. aureum* and *S. latipes* were present from
early June until late August in 1957 and thus would account for the transmis-
sion of *L. bonasae* (Table II). Transmission is likely to begin at a different
time each year as it depends on the conditions that permit enough of the suit-
able species of black flies to emerge, to become infected, and to be capable of
transmitting the infection to grouse. Owing to changeable and adverse
weather conditions the grouse chicks, especially when small, were not exposed
continually outdoors although part of the time the vectors were active. Con-
sequently, the times when the chicks became infected are more variable than
would be expected in broods of grouse that are outdoors continuously in
nature. Even when chicks are being brooded by the parent it is still possible
for the vectors to crawl beneath the feathers to transmit the parasite.

TABLE II

SUMMARY OF INFECTIONS OF *L. bonasae* OBSERVED IN GROUSE CHICKS

Grouse	Date parasites present	Approx. age (in weeks) of bird when parasites seen	Remarks
149	18/7	4	
230	27/6	2	
254	1/7	4	
263	2/8	8	
289	28/6	3	These birds developed infection while
308*	5/7	3	in captivity. The parasitaemia of
348	13/9	12	No. 254 is shown in Fig. 5
349	23/8	10	
350	30/8	9	
351	2/8	6	
361	12/9	12	
23	29/7	6	
24	29/7	6	
25	22/8	8	
195	2/7	3	These birds were infected at the time
307	2/7	3	of capture
309	6/7	5	
310*	8/7	2	

*Spruce grouse.

A low parasitaemia was seen in each of the young grouse that were kept in captivity and which were infected naturally or experimentally. A similar condition was found by Borg (2) in the capercaillie and grouse in Sweden. Moreover, neither the grouse that we kept under observation nor those killed throughout the summer (Table II) showed gross signs of disease. In view of the morbidity and mortality noted in ducks with heavy infections of *L. simondi* it should be of interest to study the composition of the blood of grouse with heavy infections. Conceivably changes would be found as Fantham (11) noted a mononuclear leucocytosis in the blood of a red grouse infected with *L. lovati*. Our qualitative observations lead us to believe, however, that *L. bonasae* is not as pathogenic as *L. simondi*. Alternatively, the failure to obtain heavy infections in grouse may be the result of many fewer simuliids feeding on grouse than on ducks.

Our grouse were kept near ducks that had intense infections with *L. simondi* but there is no evidence at present that *L. simondi* is transmissible to grouse or that *L. bonasae* will infect ducks. Rather limited evidence (7) indicates that this does not occur. Moreover, the species of flies that transmit the parasites to ducks differ from those that are suitable hosts for *L. bonasae*.

Variations in the size and shape of the gametocytes and the round and fusiform cells that contain them suggest the need for a taxonomic study of *Leucocytozoon* in various galliform birds. Conceivably differences now considered specific may be due to variation in the normal development of the parasite or be caused by the host as Clarke (3) suspected when he described *L. bonasae*. Such a study will necessitate cross infections and following the

course of the parasitaemia in various birds. In view of the widespread distribution of *S. aureum* and *S. latipes* (5, 14) it will not be surprising if they prove to be vectors of *Leucocytozoon* to grouse in many parts of the world and if so this should facilitate such a study.

Borg (2) noted in Sweden a higher incidence of *Leucocytozoon* in capercaillie than in black grouse and suspected that one bird might be more susceptible to the parasite than the other. Possibly the vectors feed more extensively on one bird than on the other. Our observations and collections of black flies that have fed on different kinds of birds indicate that the feeding habits of the vectors are important. The kind of bird, its size, and the habitat in which it lives may determine the number of suitable vectors that feed upon it. These factors together with the susceptibility of the bird to a particular parasite will affect the incidence.

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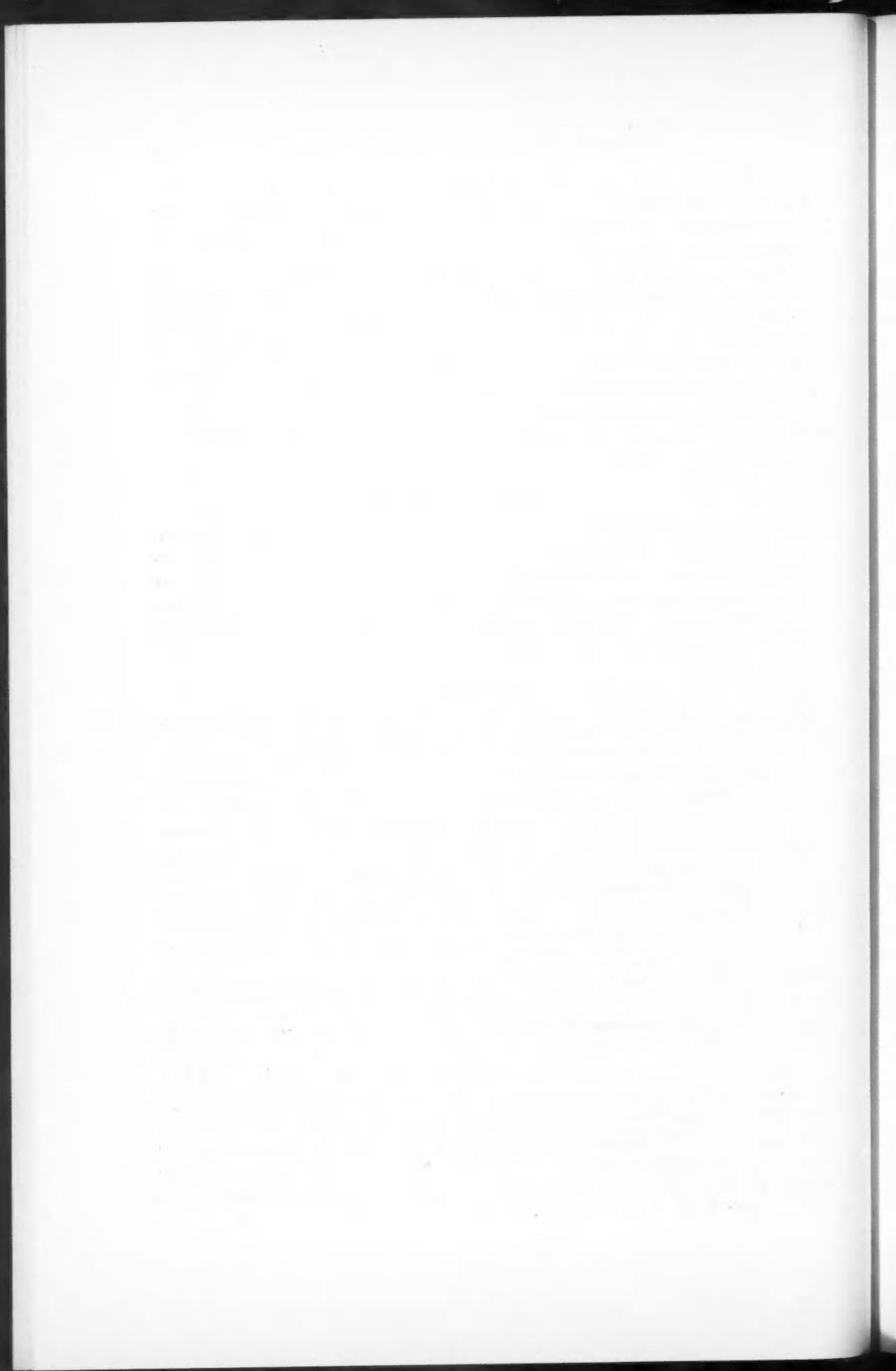
INVESTIGATIONS OF PERMEABILITY, DIAPAUSE, AND HATCHING IN THE EGGS OF THE MOSQUITO AEDES HEXODONTUS DYAR[1]

W. E. BECKEL

Abstract

Methods for collection in the laboratory of many thousands of eggs from field-collected, blood-fed females are described. Also a way of separating fertile from infertile eggs is outlined. When the eggs are laid they are white but begin to darken within one-half hour. The darkening did not occur when the egg cell or early embryo was killed.

Two changes in permeability in the prediapause stage of the eggs of *Aedes hexodontus* were observed. The first took place as the chorion changed from white to black. The egg lost and gained water easily when first laid but resisted water loss and uptake as the chorion darkened. However, a complete water-proofing did not result. With the formation of the transparent cuticle a further change in permeability occurred. The egg lost hardly any water even when exposed to extreme desiccation and what little water was lost was regained very slowly. However, the cuticle, with the chorion removed in sodium hypochlorite, was quite permeable. The impermeability of the chorion and cuticle combination must result from the bonding between the two and this bonding must be disrupted by the dechorionation.

To study the obligate diapause that occurs in the late embryogeny of the mosquito egg the effect of making water available to the embryo, of light, and of low temperature was studied. Diapause was not broken when water was available to the embryo, nor did alternate periods of light and dark at room temperature or at lower temperatures disrupt the diapause. To test the influence of cold the temperature of $1°$ and $-3°$ C. were used to simulate the temperatures in the field. The low temperatures were found to terminate the diapause but the numbers of eggs hatching did not steadily increase with an increase in the time of exposure to cold. No clue has as yet been discovered to explain these results. A hatching infusion of decaying adult mosquito bodies in distilled water was found to give more hatching than did distilled water alone. The mechanism of the hatching stimulus is not known.

Introduction

There is a paucity of information about the physiological properties of the stages of development of most mosquito eggs. This is particularly true of the common mosquitoes of northern Canada. It seemed desirable to make a systematic examination of northern species and the following observations set out some of the properties of the eggs of *Aedes hexodontus* Dyar. It is known for this species that the winter is spent in the egg stage and that eggs laid in small vials in the laboratory in the summer do not hatch if kept at room temperature even though the embryo has developed completely within. A firm obligate diapause is therefore indicated but this diapuse has not been adequately defined. Also nothing is known about the eggs from the time of laying until diapause inception or about their hatching when diapause is broken. The following experiments were, therefore, designed to provide information about the changes in the permeability of the eggs to water during the early phases of development and to determine some of the characteristics of the diapause.

Materials and Methods

Preliminary efforts were made to collect eggs for this study in their natural habitat.   The species chosen at first was *Aedes communis* (De Geer) because its larvae were found to be concentrated in large numbers in a few pools. It was hoped that the eggs might be laid in groups in specific places and that, if a clue to the location of these were found, collections of large numbers of eggs would be possible. While it was found that the eggs were laid specifically in moist places (Beckel and Barlow (13)), no large concentrations of eggs were located.   Therefore collection of suitable numbers in this way was not possible.

As collection of eggs from nature was not practical, recourse was had to collecting eggs in the laboratory.   This required that blood-fed adults be captured in the field and caged in the laboratory until eggs were laid.   For this *Aedes hexodontus* was used because the adults are very numerous and they emerge in such a manner in the Churchill, Manitoba, region, where the work was done, that at certain times during the summer in certain areas almost all the female mosquitoes attracted to man are of this species.

To collect the blood-fed females four to eight guinea pigs in wire holders were taken to the field and when 50 to 100 female *A. hexodontus* had landed on the back of the pig and begun to feed, the animal was placed inside a 2-ft. cube cage of plastic screening. The mosquitoes were allowed to complete their meal and to fly to the sides of the cage. Any specimens of banded-leg species noted on the guinea pig were removed before the animal was placed in the cage. By this precaution the only other species which might be biting the animal would be other dark-legged species such as *Aedes nigripes* Zetterstedt or *Aedes impiger* (Walker), which occur in small numbers in the locale selected for collection, and *Aedes cinereus* Meigen, which is a rare mosquito in the Churchill region.   Identification of samples of females from the cages, using a key for rubbed specimens (Beckel (11)), showed these to be almost entirely *Aedes hexodontus*.   When 500 to 1000 engorged females were in the cage it was transported to the laboratory.

Two methods of collecting eggs were used. In the first method the females were left in the 2-ft. cube cages and were offered bags of water-soaked raisins as supplementary food.   The cage was placed on an oviposition site of wet cotton sheeting over wet absorbent cotton with the screen touching the cotton.   In 5 to 7 days many thousands of eggs were laid on the wet sheeting through the screen.   The air temperature of the room was $20 \pm 2°$ C. At any time the cage could be removed from the oviposition site without disturbing the mosquitoes and the sheet and eggs taken away.   The eggs were allowed to remain on the wet sheet undisturbed for 15 days.   In preliminary tests it was discovered that drying or handling of the eggs, particularly in the first few days following laying, caused excessive mortality of the embryos. After 15 days the embryos had developed fully but had gone into diapause. The eggs could then be handled and stored even in relatively dry conditions without apparent damage.

The second method was used because the method described above was not convenient for obtaining eggs of known history from a few positively identified females. A special oviposition chamber was set up as follows (Fig. 1). A lantern globe was closed at the top with cheesecloth taped to the glass or held with an elastic band. In the center of the cloth a small slit was cut. A square cardboard or cork plaque larger than the top opening of the globe was centrally pierced with a No. 3 insect pin on which were impaled four or five water-soaked raisins. If a liquid nutrient was to be fed a modification described by Barlow (4) using an absorbent cotton filled glass tube was inserted through the plaque. In either situation the plaque was placed on the top of the globe with the pin and raisins or the glass tube projecting through the slit in the cheesecloth into the globe. A petri dish was packed slightly higher than the top with wet absorbent cotton and covered with a filter paper. A square of plastic screening larger than the petri dish was stiffened by stapling it to a plywood frame and was placed over the dish so that the screen touched the filter paper. Stiff wire screen with no frame was also used with no ill effect (Fig. 1). The lantern globe assembly was placed on the screening directly over the petri dish. Blood-fed female mosquitoes from the field were collected as described for the first method but groups of these were identified in the tube of an aspirator with a binocular microscope. The aspirator tube was then placed through the slit in the cheesecloth top of the lantern globe, the mosquitoes gently blown into the globe, and the
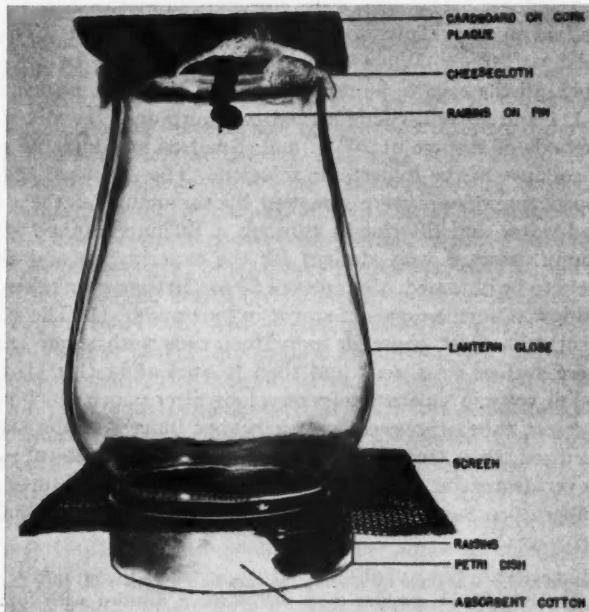
FIG. 1. Oviposition chamber for method 2.

slit closed with the plaque. The supplementary food was changed or added daily and the cotton in the oviposition site kept wet. From 1 to 25 adults could be maintained satisfactorily. At 20° C. eggs were laid through the screen on the wet filter paper in 5 to 7 days. With 25 adults present the globe and screen as a unit was lifted every one-half hour at the peak of oviposition. The filter paper with fresh eggs was removed, labelled, and stored on a wet substrate. In this way a timed series of eggs could be obtained. Over 60% of the eggs laid in these chambers were fertile and developed. An average of 30 eggs per female was obtained with 25 females to a chamber.

All the experiments on permeability of eggs were done at a temperature of $20 \pm 2°$ C. This was also the temperature for the experiments to test the effect of water on diapause and for some of the experiments on the effects of light. Unless specifically mentioned, groups of 25 to 100 eggs were used for each experiment. To test the effect of cold on diapause, temperatures of 1° C. and $-3°$ C. for exposures of approximately 200 days were used. The temperatures and time of exposure were chosen to simulate field conditions. In the Churchill, Manitoba, region the winter lasts a period of 6 to 7 months or approximately 200 days. The temperatures in the field during the winter in places where the eggs are normally located vary from 1° C. to $-3°$ C., as determined by thermocouple readings in soil and water typical of mosquito breeding pools (for subsurface temperatures see Beckel, D. K. B. (9)).

For the experiments with the influence of cold on diapause the procedure used was as follows. The eggs were gathered from the 2-foot cube cages. Some were laid in early July, some as late as mid-August. They were all stored, moist, at 20° C. When the experiments were to be set up, usually in September, all the eggs to be used were combined in a beaker of glycerin and water 1:1 and stirred. This served two purposes. It mixed eggs with different periods of storage at 20° C. and it caused any infertile eggs to lose water and collapse in the hypertonic solution. The collapsed eggs sank and the developed turgid eggs were removed by siphoning off the top layer of glycerin and water and filtering it through a Büchner funnel. Only fertile eggs randomly assorted were desired for the experiments and this method enabled them to be obtained. Samples of 50 to 250 eggs were taken at random from the supply of fertile eggs and stored in four ways. (1) The samples were placed directly on filter paper in individual vials with screw caps; (2) the samples were surface sterilized* and then treated as in (1); (3) the samples were placed directly in separate piles on a long filter paper which was inserted into a long test tube stoppered with a rubber bung; (4) the samples were surface sterilized, then treated as in (3). The vials or test tubes were then placed at ever-decreasing temperatures over a 7-day period until the experimental temperature was reached. Many replicates of each method of storage were set up.

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* Surface sterilization was done by placing the eggs in a solution of 10% NaOH and 5% formalin for 15 minutes, then washing them well in sterile distilled water. The efficacy of the method was proved by plating eggs on nutrient agar. No apparent harm resulted to the embryos within the eggs.

After two weeks to a month had passed and approximately two weeks thereafter a trial of hatching was attempted by removing one sample of 50 to 250 eggs from each experiment and placing the eggs in a hatching medium. The hatching medium was either distilled water or a special hatching infusion (Beckel (10)). The hatching infusion was a mixture of ground adult mosquito bodies in distilled water, allowed to decay. The eggs from the cold were placed in hatching medium at 1° C. at first and the temperature was allowed to rise to 20° C. over a 24-hour period. Our experience showed that if hatching was to occur it did so within five days at 20° C. Therefore after five days the eggs were discarded. They were not returned to their experimental temperature for further attempts at hatching. In all trials evidence of breaking of diapause was a hatch of over 2% to produce viable larvae. A hatch of up to 2% was considered arbitrarily to have occurred by chance.

Results

Chorion, Cuticle, and Permeability in the Prediapause Egg
Chorion

The chorion of *Aedes hexodontus* eggs was like the chorion of *Aedes* eggs reported by other authors (de Buck (15), Harwood and Horsfall (19)). It consisted of two layers. The outer layer, the exochorion, was a pebbled, transparent wax-like layer; the inner layer, the endochorion, was a pliable material which turned from white to black within two hours of oviposition. This finding is similar to that observed for *Aedes aegypti* L. by Gander (17). The first appearance of darkening was noticed after one-half hour in most of the eggs laid but a few always remained white and none of these developed. This suggested that the egg must be alive to be able to darken and led to experiments to determine if killing the egg cell of fertilized or unfertilized eggs would stop darkening. When freshly laid eggs from mated or unmated females were subject to heat (90° C. for 1 minute), drying (desiccation over $CaCl_2$ for 2 hours), or to cyanide (0.1 M KCl, left in the solution) they did not darken. These results then support the hypothesis. As suggested by Gander (17) the reaction may be a tanning process, possibly a typical melanin formation with tyrosinase acting on tyrosin to produce melanin. However when white eggs (freshly laid) from mated or unmated adults were placed in a saturated solution of polyphenol oxidase inhibitor phenylthiourea (P.T.U.) they blackened normally and those from mated females went on to develop. As mentioned potassium cyanide (0.1 M) effectively inhibited the color reaction but none of the eggs developed hence the cyanide may not have had a specific effect. Also the P.T.U. may not have penetrated the exochorion as did the cyanide.

The permeability to water was tested by placing newly laid eggs in a desiccator over $CaCl_2$. Within moments they began to collapse indicating water loss. When returned to water they rapidly regained their shape. When white eggs were placed in absolute alcohol they were fixed within seconds without change in shape. However as the eggs began to blacken in the

normal way they became partially collapsed when placed in absolute alcohol although ultimately they were fixed as the alcohol entered. In hypertonic salt solution slightly darkened eggs also collapsed as if water was being extracted from them. When placed back in distilled water the eggs regained their turgor but more slowly than it was lost. It appears that their permeability had changed. Such a change in permeability has been reported for *Aedes aegypti* eggs by Gander (17). To investigate further this change in permeability recently blackened eggs were killed by treatment in water at 90° C. for 1 minute and placed in hypertonic saline. They collapsed in the saline and regained their turgor in distilled water as rapidly as viable eggs. Apparently neither heat nor death affects the permeability as far as has been determined at this stage, unless as a consequence of heat coagulation the osmotic pressure of the yolk was increased just enough to offset a decrease in permeability.

Cuticle

In some insect eggs, after the serosa of the embryo has been formed, a new envelope is secreted around the embryo by the serosal cells (Slifer (25)). This cuticle is usually secreted against the vitelline membrane which is itself in close contact with the chorion. In the egg of *Aedes hexodontus* the cuticle is laid down after approximately 48 hours at 20° C. and is transparent. This was determined by dechorionating eggs allowed to develop for 30 to 60 hours after oviposition. To dechorionate the eggs they were placed in a saturated solution of sodium hypochlorite (Slifer (26)) which dissolves both layers of the chorion in 5–10 minutes. Most of the eggs less than 48 hours old completely disintegrated in sodium hypochlorite; most of the eggs more than 48 hours old showed a transparent cuticle resistant to the hypochlorite. When cross sections of eggs 46, 48, and 50 hours old were examined a layer of serosal cells just under where the cuticle was forming was seen. It is assumed that this produced the cuticle. Some authors (Telford (29)) call this extraembryonic envelope the vitelline membrane, others refer to it as a fertilization membrane (Beament (8)).

Following cuticle formation the intact eggs no longer collapsed in hypertonic solutions even when exposed for 18 hours. Also intact fertile eggs which had developed for at least 48 hours did not collapse in a desiccator over $CaCl_2$ even when left for 5 days. Some did become slightly indented in a few hours and a few became more extremely indented; but when later dissected even the extremely indented eggs had viable embryos within. However, eggs with a well-developed cuticle, punctured with a fine needle to destroy the integrity of the chorion and cuticle, collapsed completely in the desiccator in a few hours, and the embryos were dead. When intact indented eggs were returned to distilled water they absorbed moisture slowly but took up to 14 days to regain their original shape. The punctured eggs when placed in distilled water swelled within a few hours and the dead embryos burst out. It is evident that with the appearance of the cuticle a second change in the permeability of the eggs occurs. An almost complete waterproofing results.

The relation of the cuticle to this waterproofing of *Aedes* eggs was reported by Beckel (12) and has recently been confirmed by Telford (29). It was noticed in the eggs of *Aedes aegypti* by Gander (17) but does not seem to have been associated with cuticle formation.

Since the change in permeability occurred after the formation of the cuticle it seemed logical to suspect that the cuticle had imparted the impermeability. The properties of the cuticle were therefore of interest. The method of dechorionation with sodium hypochlorite permitted a test to be made of the permeability of the cuticle alone. When dechorionated eggs were placed in water or absolute alcohol they ruptured in water after from one to five hours and were fixed in alcohol within one hour. Therefore, although the chorion plus the cuticle renders an egg relatively impermeable, the cuticle dechorionated in hypochlorite is permeable. The bonding of the cuticle to the chorion may be what is responsible for the impermeability of the chorion–cuticle combination and this bonding may be disrupted by the hypochlorite.

No detailed information on the chemical nature of the chorion or cuticle or the bond between them is available. Some authors have suggested that the chorion of *Aedes aegypti* contains chitin (Atkin and Bacot (2)). But in *Anopheles*, Nicholson (24) shows that the chorion will dissolve in potassium hydroxide and so only resembles chitin. A test for chitin in both the isolated chorion and the cuticle was made. The chitosan test as outlined by Gatenby and Beams (22) was used. The chorion alone, without the cuticle or its bonding to the chorion, was obtained by using infertile eggs. Such eggs lack a cuticle, only the vitelline membrane surrounding the yolk under the chorion being present. Many such eggs gave a consistently negative test for chitin. To test the cuticle alone, the chorion, vitelline membrane, and probably the bonding of the cuticle to the chorion was removed in sodium hypochlorite from many eggs which had developed at least 100 hours. The cuticle then remained surrounding the developed embryo. The dechorionated eggs were then left in distilled water until they had ruptured; the embryo then swelled out of the cuticle. The embryos were removed, leaving the empty cuticles. When the latter were tested for chitin the test was consistently positive.

The appearance of chitin seems to be correlated with the change in permeability of the egg but the chitin is a part of the cuticle and the cuticle, as shown, is permeable. Therefore, the chitin is not responsible for the impermeability unless as it is formed in the cuticle it also plugs holes in the chorion or in the cuticle–chorion bonding and these latter are removed or disrupted when dechorionation takes place.

The effects of death of the embryo and of heat on permeability of intact and dechorionated eggs with well-developed cuticles were tested in a manner similar to that with eggs before cuticle formation mentioned earlier. Intact eggs with a cuticle and chorion around the embryo, when placed in water at 90° C. for 1 minute, did not collapse any more rapidly in a desiccator over CaCl₂ than did non-treated eggs. Nor did treated desiccated eggs take up water any more rapidly than non-treated eggs when left in distilled water

for over 24 hours.   Dechorionated eggs with just a cuticle around the embryo,
when exposed to water of 90° C. for 1 minute, began to rupture in the hot
water and broke open in cold distilled water within one minute.   One can
tentatively conclude that death of the embryo or treatment at 90° C. had
no effect on permeability of the intact egg membranes, unless the heat changed
the osmotic pressure inside the egg just enough to offset the permeability
change.   However, the heat treatment and consequent death of the embryo
changed the transparent cuticle, apparently making it more permeable than
it normally is.

Dechorionated eggs with their permeable cuticles were placed in a solution
of trypan blue stain to see if any specific area of water entrance could be
traced by the stain.   The eggs were left in the stain for one-half hour, then
removed and placed in distilled water where they ultimately ruptured and
the embryo swelled out of the cuticle.   A small ring of blue stain was noticed
on the transparent cuticle only in the region where the hatching spine of the
enclosed embryo had been.   It looked as if the hatching spine had pushed
through the cuticle allowing entrance of water and stain.   Hypothetically all
evidence of water passage through the cuticle might be attributed to a hole
made by the hatching spine.   This hypothesis was proved false by the following
experiments.   Eggs were dechorionated after they had a well-developed
cuticle but before a hatching spine had developed in the embryo.   When
these were tested in water at 20° C. or at 90° C. they showed the same perme-
ability as dechorionated eggs where the embryo had a fully developed hatching
spine.   As further evidence dechorionated eggs containing embryos with no
hatching spines were placed in a desiccator of $CaCl_2$; within a half hour they
had indented slightly.   They were then placed in trypan blue for one-half hour
and they took up moisture until most had regained their full form and some
had burst.   All were then removed and placed in distilled water until the
remainder burst and the embryos swelled out of the cuticle.   The cuticle
was permeable in itself, not as a result of a hole made in it by a hatching spine.

It was noted that on the cuticle of the eggs which had become turgid in
the trypan blue but had not burst there was no sign of stain.   But on the
cuticle of eggs which had burst in trypan blue a blue ring around the point
of rupture was observed.   The cuticle was therefore permeable to water and
not to the stain and only the inside of the cuticle took the stain.   However
the sodium hypochlorite may have altered the outside of the cuticle so that
it would not take the stain whereas the normal inside did.   If this is not the
case, then the outer and inner surfaces of the cuticle must be different chem-
ically.   This suggests that the cuticle is two-layered as has been found in the
grasshopper egg by Slifer (25).

It is now evident that there are permeability changes before and after forma-
tion of the transparent cuticle.   Work with *Rhodnius* egg chorion (Beament (6))
shows that a primary wax layer, laid down by the oöcyte on the inner surface
of the endochorion, is responsible for early impermeability.   Slifer (28) shows
that such a layer has a similar function in the grasshopper egg.   It is con-
ceivable that a primary wax layer may well be formed on the endochorion

of the mosquito egg and account for the change in permeability during blackening. As mentioned for *Rhodnius* the oöcyte would be responsible for laying down this layer because the change in permeability does not occur if the oöcyte is killed. However, along with the change in permeability blackening occurs and it may be that the chemical changes associated with blackening simply cause an increased resistance to water passage accounting for the change in permeability. This is suggested to account for the initial permeability change in *Aedes aegypti* eggs by Gander (17).

Beament (7) also described for *Rhodnius* the presence in the cuticle of a waxy substance which makes the cuticle a second waterproofing layer. This wax is laid down by the embryo. In the grasshopper a similar second waterproofing layer is provided by a layer of wax between the chorion and the cuticle (Slifer (28)). The true waterproofing of the mosquito egg, which takes place when the cuticle is formed, may involve a wax; but if so this wax must somehow be concerned with the bonding of the cuticle to the chorion; the cuticle alone after dechorionation with sodium hypochlorite is permeable.

Diapause and Hatching

In the field when the fertile egg of *Aedes hexodontus* is laid, if it is in a moist environment, it begins to develop immediately and continues until the embryo is just about to hatch. At this stage morphogenesis ceases and the egg enters a firm obligate diapause. Laboratory experiments indicate that normal development will ensue even if the egg is covered with water. Inundation of most pools where *A. hexodontus* eggs are laid occurs within a few weeks of the cessation of laying, as the autumn rains fill up the pools. Most of the eggs spend the autumn under water and the winter under the ice. In the spring they are still submerged and with no exposure to air hatch when the water temperature rises a few degrees above zero Centigrade. In the field, diapause in *Aedes hexodontus* eggs is presumably broken after exposure to a temperature low enough to inhibit morphogenesis but to permit diapause development (Andrewartha and Birch (1)). However, diapause in some insects is broken by stimuli other than low temperatures. Slifer (27) has found with grasshopper embryos that diapause can be broken by making water slowly available to the embryos. She placed the eggs in a fat solvent, xylene, for short periods which made them more permeable and then transferred them to moist filter paper. Water was slowly taken up and development resumed. Also light has been found to break the diapause of the treehole mosquito *Aedes triseriatus* Say (Baker (3)). Experiments were therefore performed to test the effects of water, light, and low temperatures on the diapause in *Aedes hexodontus*.

Three attempts were made to make water available to mosquito embryos. First eggs with fully developed embryos in diapause were abraded with a sand and water mixture agitated for 24 hours. The criterion for diapause breaking was taken to be hatching of the eggs. Even many days after the abrasion treatment no hatching occurred. However, the actual effect of the

treatment on water entrance through the chorion and cuticle was not deter-
mined. A second experiment involved placing diapausing eggs in solutions
of fat solvents, such as xylene, benzene, acetone, naphtha, ether, and ethyl
alcohol, for varying periods of 2 minutes to 48 hours. The eggs were then
placed on moist filter paper or directly into distilled water. If the eggs stayed
in the fat solvents long enough the permeability of the egg membranes was
clearly affected because the eggs split in contact with water but dead embryos
swelled out. Shorter treatments showed no effect of the fat solvents on
water uptake and no hatching or rupturing resulted, even after many days.
However, subsequent dissection of the eggs revealed live embryos within.
Dechorionated eggs were also treated with fat solvents. The solvents rapidly
changed the permeability of the transparent cuticle so that water entered
within minutes, many times more rapidly than with no fat-solvent treatment;
but the embryos in this case were found to be dead. This indicates that a
fatty substance may be present in the cuticle but it does not act as a complete
waterproofing agent, at least after dechorionation in sodium hypochlorite.
Finally, fully formed embryos in diapause were dissected from eggs, alive and
apparently uninjured, and placed in distilled water, insect Ringer (Ephrussi
and Beadle (16)), and in a tissue culture medium, M150 (Morgan et al. (23)).
In all the solutions some embryos began to move their mouth parts, show
heartbeat, and to absorb fluid from their tracheal system. But in from 2 to
72 hours all swelled and died. In normally hatched larvae, which have
broken diapause, the head capsule fills with fluid, becomes wider than the
thorax and abdomen, and darkens. This never happened with an embryo
dissected from the egg before diapause development was complete and morpho-
genesis had commenced.

To test the effects of light on diapause fully developed *Aedes hexodontus* eggs
conditioned at room temperature for over 30 days were exposed to light at
various temperatures. Light from 60-, 25-, and 15-w. tungsten filament lamps
and 15-w. fluorescent lamps was used. All lamps were placed 12 in. from
the vials of eggs. The experiments involved daily exposure to 18 hours of
light and 6 hours of darkness for periods of 10, 15, and 21 days. Eggs at
20° C. with no exposure to cold were treated. Also treated were eggs which
had been at 7°, 4°, 1°, and −3° C. for a few weeks to 6 months. Controls
were run in complete darkness. Temperatures inside the vials were recorded
with thermocouples. When the treated eggs and controls were tested for
hatch, no hatching resulted in the eggs kept at room temperature; there was
some erratic hatching in the eggs exposed to cold. But the hatching could
not be definitely correlated with exposure to light. The above conditions
of light alone or in combination with the exposure to cold do not terminate
diapause in *Aedes hexodontus*.

The effects of temperature were studied to decide whether cold would break
diapause and if so to define a time course of breaking of diapause by cold.
A large number of experiments were performed, some in different years. The
temperatures as mentioned above were 1° C. and −3° C. for approximately

200 days of exposure. The procedure followed in these experiments is outlined in Materials and Methods but generally involved putting unsterile and surface-sterilized eggs in vials or test tubes at cold temperatures and making a trial of hatch with a sample of 50 to 250 eggs taken from each experiment usually starting after a month's exposure and every 2 weeks thereafter. The hatching medium was either distilled water or an infusion of decaying mosquito bodies in distilled water (hatching infusion).

As the experiments proceeded it was found that the numbers hatching from any experiment did not show a steady increase as time in the cold increased. The numbers hatching from trial to trial were in fact quite erratic. For example at $-3°$ C., after 98 days' exposure, hatching was 13%; at 112 days, 8.8%; at 127 days, 77%; and at 140 days, only 13%. Each of these trials had over 100 eggs. It is evident that there were factors totally unrecognized which frequently made the individual samples of eggs, supposedly identical except for time of exposure to cold, different from one another. Each experiment is therefore best considered by combining the results of all the trials into only two groups, hatching in the first 100 days of exposure, and hatching in the second 100 days of exposure. The median percentage hatch and the range is given for various experiments in Table I. The data show that there were always more eggs hatching in the second 100 days of exposure to cold than in the first; they also show that the hatching infusion was a better hatching medium than distilled water.

TABLE I

PERCENTAGE HATCH OF MOSQUITO EGGS EXPOSED FOR 200 DAYS TO VARIOUS TEMPERATURES

	0–100 days		101–200 days	
Treatment	Median % hatch	Number of trials, 50 to 250 eggs per trial	Median % hatch	Number of trials, 50 to 250 eggs per trial
1° C., hatched in distilled water	5.0 (0–54)	2* and 5	11.0 (0–38)	3* and 4
1° C., hatched in hatching infusion	26.7 (16.6–46.7)	5	48.5 (32.6–78.1)	4
−3° C., hatched in distilled water	7.1 (0–63.6)	3, 5, 7, and 6	32.5 (2–77.6)	6, 6, 7, and 7
−3° C., hatched in hatching infusion	9.1 (0–49.2)	5, 7, 7, 6, and 6	57.9 (0–95.7)	6, 7, 7, 7, and 7
−3° C., eggs surface sterilized, hatched in distilled water	2.5 (0–34.9)	7 and 6	14.8 (0–34.7)	7 and 7
−3° C., eggs surface sterilized, hatched in hatching infusion	8.1 (0–24.9)	7 and 6	55.2 (25.7–76.3)	7 and 6

* Each number represents trials from a different experiment.

As a control for the experimental eggs exposed to cold thousands of eggs were kept at room temperature and hatching attempts were made throughout 200 days. At no time did more than 2% of the eggs hatch. The conclusion seems justified that diapause can be broken by cold but that other unknown factors also are involved.

The literature throws some light on the results. Borg and Horsfall (14) and Horsfall (20), working mainly with summer floodwater species of mosquitoes, point out the importance of proper conditioning of the eggs before hatching is attempted. They feel that if the conditioning is adequate then a lowering of the dissolved oxygen of the hatching medium will cause hatching. In the present experiments with *Aedes hexodontus* the conditioning is a close approximation of what happens in the field so is probably adequate. But low dissolved oxygen in the hatching medium may be a necessity and the hatching stimulus used with the *Aedes hexodontus* eggs may have had too much dissolved oxygen to produce maximum hatch in every instance. If this conclusion is to explain our erratic hatching then the level of dissolved oxygen in the hatching medium must be very critical and would have fluctuated slightly in different hatching attempts even though every effort was made to equate the experimental procedures from one hatch attempt to another. Borg and Horsfall (14) and Horsfall *et al.* (21) do mention that the amount of dissolved oxygen is critical. They report different amounts of hatching with different degrees of oxygen reduction. Certainly there would be a lower amount of dissolved oxygen in the hatch infusion with its high bacterial population than in the distilled water. This could account for the generally better results obtained with hatch infusion. However the effect of low oxygen concentration on hatching of mosquito eggs is only claimed for floodwater and particularly summer species. There is scant evidence of its effect as a stimulus for a spring species and particularly one such as *Aedes hexodontus* that is not a typical floodwater species.

Tests of the water in which *Aedes hexodontus* hatches in the spring shows it, in most instances, to be nearly saturated with dissolved oxygen. Also experiments with conditioned *Aedes hexodontus* eggs have been tried where they were placed in actively growing bacterial cultures in extract of canned corn or beef heart extract and in distilled water over pyrogallol for up to 15 days. All of these media would presumably have low amounts of dissolved oxygen. No hatching resulted. More such experiments might be done with more attention being paid to exactly equating and perhaps changing the conditioning of the eggs and to exposing them for hatch in water where the manner of removing the oxygen is varied. Horsfall (20) has reported that the time when the oxygen is removed from the hatching medium as well as the amount removed is critical with floodwater species.

Another factor which could influence our results is the discovery by Gillett (18) of an inheritable difference in ability of *Aedes aegypti* eggs to respond to hatching stimuli. Such an inherited difference in hatching response might account for the erratic hatching obtained in the results with *Aedes hexodontus*.

However the genetic variation should have been removed from our experiments by the procedure wherein the eggs to be used were mixed and the samples for each experiment were chosen at random.

Acknowledgments

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THE MANNER IN WHICH THE SPONGE CLIONA BORES IN CALCAREOUS OBJECTS[1]

Frederick E. Warburton[2]

Abstract

Boring in shells and calcite crystals was investigated, using a new type of reconstitution culture. No free acid was detected, and no detectable quantity of calcium was put in solution by the sponge. However, acid action is indicated by restriction of boring to calcium carbonate. Conchiolin is penetrated with difficulty. Cells in contact with the substratum form a reticulum of fine pseudopodia and filaments; a corresponding pattern of lines is etched into the calcite. The areas so marked out are the same size and shape as the fragments discharged by the sponge. These fragments are identical whether from shells or calcite crystals; their faces are curved but their edges sharp. Apparently the cytoplasmic filaments insinuate themselves into calcite by secreting minute amounts of acid, and undercut fragments which are carried through the sponge parenchyma to excurrent canals.

Cliona is a cosmopolitan genus of marine siliceous sponges (F. Clionidae, O. Hadromerina, Cl. Demospongiae), remarkable for the habit of living in tunnels and galleries bored in limestone, coral, and the shells of molluscs. The damage inflicted by *Cliona* on various mollusc fisheries and on limestone breakwaters invests the genus with some economic importance, and sponge-riddled shells and stones are familiar objects to the naturalist on the seashore. The manner in which the galleries are excavated has therefore attracted the researches of several biologists, and the speculations of many more.

This paper will recount some of the results of an investigation into the biology of boring sponges, sponsored by the Fisheries Research Board of Canada because of the deleterious effects of the sponges on the oyster and scallop fisheries of eastern Canada. The work was carried out at the Board's Biological Sub-Station, situated on Malpeque Bay on the north shore of Prince Edward Island, near Ellerslie. Studies were largely confined to *Cliona celata*, the prevalent species in that area.

Historical Note

Grant (3), who first described *Cliona celata*, believed that his new "zoophyte" dwelt in burrows made by worms. Bowerbank (1) shared this opinion. Hancock (4,5) refuted Bowerbank's arguments, and made it clear that *Cliona* bored its own holes; he suggested that this was done by grinding with the spicules. However, Nassanov (9,10) showed that *Cliona* larvae began to bore before they possessed spicules.

Nassanov had described the fragments of shell discharged by boring sponges. These, and fragments of conchiolin from bored oyster shells, were figured by Topsent (12), who pointed out that since conchiolin is bored in the

same way as shell it is difficult to accept the hypothesis that *Cliona* bores by secreting an acid.   Hancock had felt that a simple organism like *Cliona* could not be expected to perform complex chemical feats like the secretion of acids.   Letellier (6) found that the tissues of *Cliona* are not acid, and pointed out that the detached fragments of shell are not corroded at the edges. He proposed that contractions of the sponge tissues might set up torsions which would break off bits of the shell to which they stuck; such torsions experimentally set up with rubber rods broke off lunular fragments resembling, but larger than, those produced by *Cliona*.   His hypothesis failed to receive general acceptance.

Vosmaer (13) reviewed the early work on the problem, and suggested seeking an explanation by comparing the action of *Cliona* with that of various boring algae and fungi.   He also suggested that the sponge secretes an acid which dissolves calcium carbonate, and, in addition, an enzyme which attacks conchiolin.   Otherwise, he was unable to do more than confirm the earlier work of Nassanov and Topsent.

Galtsoff and Pertzoff (2) titrated suspensions of the cells of *Cliona* and of *Microciona*, and found that the *Cliona* suspension bound less acid and more base than the other.   Old (11) developed the extremely useful technique of cultivating *Cliona* larvae on crystals of calcite.

Since the time of Nassanov and Topsent, however, little has been published on the boring mechanism of the Clionidae except speculation—often dogmatic speculation.   It has been suggested that the boring is done by an alga or fungus symbiotic with the sponge; but this seems to be simply a retreat from the problem.   No constant association of sponge and fungus has been demonstrated; and the few boring fungi which the writer has observed in his cultures have formed rectangular reticula, which could not remove fragments of calcite shaped like those discharged by the sponge.   Another suggestion was that *Cliona* does not secrete acid, but attacks shells solely by enzymatic action on the conchiolin which is supposed to bind them together; but the sponge bores perfectly efficiently in limestone and pure calcite.   It has even been stated that "*Cliona sulphurea*" (a synonym of *C. celata*) bores by secreting sulphuric acid; a hypothesis with no support but linguistic association.

Materials and Methods

Spongy shells of oysters, *Crassostrea virginica*, and of other molluscs, were obtained by dredging infested oyster beds.   These sponges could be kept alive indefinitely in an aquarium with constantly flowing sea water, as stock material for the study of boring.

I adopted Old's idea of culturing sponges on transparent calcite crystals. However, instead of waiting for the irregular and uncertain supply of sponge larvae, I used "reconstitution cultures".   When spongy shells are broken up with bone-cutting forceps, they tend to split horizontally into large flakes, exposing masses of sponge which can be scraped off with a blunt scalpel or forceps.   Fragments up to about 0.05 ml. in volume could be obtained in

this way.   Such pieces were dropped into a finger bowl of filtered sea water for a few minutes, to remove loose cells which would die and foul the cultures, and then transferred by pipette to a calcite crystal, cover glass, or other substrate, in a second finger bowl.   If large sponge fragments could not be procured, several small ones were piled together.   The cultures had then to be left undisturbed for three or four days, without a change of water. After that time, the fragments had attached themselves to each other and to their substrate well enough to permit them to be transferred daily to new water in clean dishes.

Within two or three weeks these masses of sponge tissue had flattened themselves into discs about a millimeter thick at the center, tapering to a single cell at the edges.   The largest such discs obtained were about 15 mm. in diameter, and quickly developed a complicated system of excurrent canals with oscula.

If paraffin sections were required, reconstitution cultures were removed from calcite crystals by dropping crystal and sponge into Bouin's fixative. Within 24 hours the sponge floated off the crystal, and could be embedded in paraffin and sectioned in the usual way.   Calcite fragments in the specimen were destroyed by the fixative and spicules did not make sectioning difficult, although frequent knife-sharpening was necessary.   After considerable experimentation, I chose Heidenhain's iron haematoxylin followed by eosin as the most generally useful stain.

For microscopic study of the early stages of the boring process I needed to remove sponges from their calcite without damaging the crystal surface; and to examine the cells in contact with the substratum, I had to remove sponges from glass cover slips on which they were growing in such a way as to leave a single layer of cells behind.   I tried a variety of elegant methods, until I found that I could obtain good results by gently wiping off the sponge with my thumb.

To study the gross pattern of sponge borings in shells, wax casts of the tunnels were prepared.   Previous workers have depicted borings that are exposed by breaking away surface layers of shell (Hancock, copied by Vosmaer (14); and Medcof (8)), by grinding sections of bored objects (Vosmaer (14)), by photographing thin translucent shells (Medcof (8)), or by using X-ray photographs (L. M. Dickie and P. Ghent, unpublished).   None of these methods give a clear three-dimensional view of the sponge cavities.

Shells were prepared for tunnel castings by boiling in sodium hydroxide solution until free of sponge tissue, rinsing in boiling water, and draining until the cavities were almost, but not quite, empty of water.   They were then infiltrated with paraffin kept melted in a boiling water bath.   They were alternately subjected to sufficiently reduced pressure to boil the remaining water out of the tunnels and to atmospheric pressure to force the melted paraffin into the cavities evacuated by the steam.   When the paraffin had hardened, it was scraped from the outside of the shells with a knife and wire brush, and the shell was dissolved in hydrochloric acid.

Certain experiments involved analyzing sea water in which sponges had been kept, for its content of dissolved calcium. The sea water from each experimental container was filtered and divided into two or more parts, each of which was analyzed by the usual method of precipitation with ammonium oxalate solution, re-solution in sulphuric acid, and titration with standard potassium permanganate solution.

Results

Reconstitution cultures could be grown on any clean surface which did not release toxic solutes into the water. They readily excavated cavities in several species of mollusc shells, limestone, and calcite crystals, but completely failed to penetrate the surface of any of the following substances: sandstone, brick, china, concrete, glass, bakelite, black rubber, hard rubber, celloidin, wood, paraffin wax, crab shell, and conchiolin. (The last substance was obtained in sheets inside the shells of oysters kept alive several weeks after their hinges had been dislocated.)

Cultures of *Cliona* on calcite were immersed in a variety of indicator solutions in sea water and examined under the high power of the microscope for signs of localized regions of low pH. No such signs were observed. Other cultures on cover glasses were inverted over depression slides filled with indicator solutions, and the thin edge of the culture observed under oil immersion; again, no color changes indicative of acid were seen. One weakness of these tests is that all indicators used were toxic in the high concentrations needed to make color changes visible in microscopic volumes. Their toxicity may have arrested those very processes they were expected to reveal. That they were toxic is shown by the fact that sponges exposed to them for an hour or two died a few days after. A critic of this report has pointed out another weakness which would have obtained even if the indicators had not been toxic. In the presence of carbon dioxide, calcium carbonate tends to become soluble bicarbonate. The reaction is reversible and the equilibrium delicate. This means that, even in the general alkaline medium of sea water, sponges may be able to produce sufficient carbon dioxide at localized sites to convert carbonate into soluble bicarbonate and erode shell or calcite. It also means that pH's at sites of erosion, in surrounding media, and in sponge tissues may all be on the alkaline side of neutrality and that differences between these pH's may be very slight indeed. If they are detectable at all, one should expect them to be revealed only by refined techniques, not by the crude methods of microscopic inspection I have depended on.

Many spicules are included in the sponge fragments used for reconstitution cultures, but these become arranged around the edge of the discs, with their points radiating outward and not in contact with the substrate. Boring may begin anywhere within the disc, and is not confined to regions containing spicules. Transverse sections of actively boring sponge cultures confirmed that no spicule points were directed toward the calcite. Also, I was able to rear a number of sponge larvae through metamorphosis on calcite crystals.

PLATE I
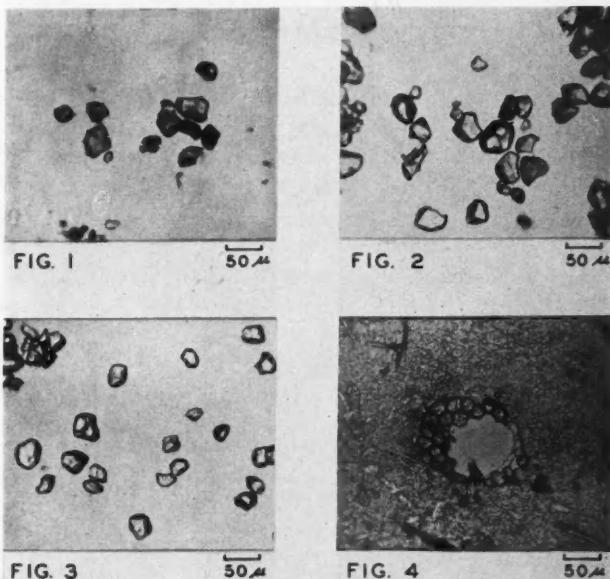
FIGS. 1–3.   Views with transmitted light of unmounted fragments disgorged by *Cliona celata* boring in, (1) oyster shell; (2) quahaug shell; (3) calcite crystal.
   FIG. 4.   Sheet of conchiolin obtained by dissolving a spongy oyster shell in hydrochloric acid, showing a perforation, with incompletely detached marginal fragments, made by *Cliona celata*.   Unstained; mounted in balsam; photographed by transmitted light.

PLATE II

FIG. 5   50 μ   FIG. 6   50 μ

FIG. 7   50 μ   FIG. 8   50 μ

FIG. 9   50 μ   FIG. 10   50 μ
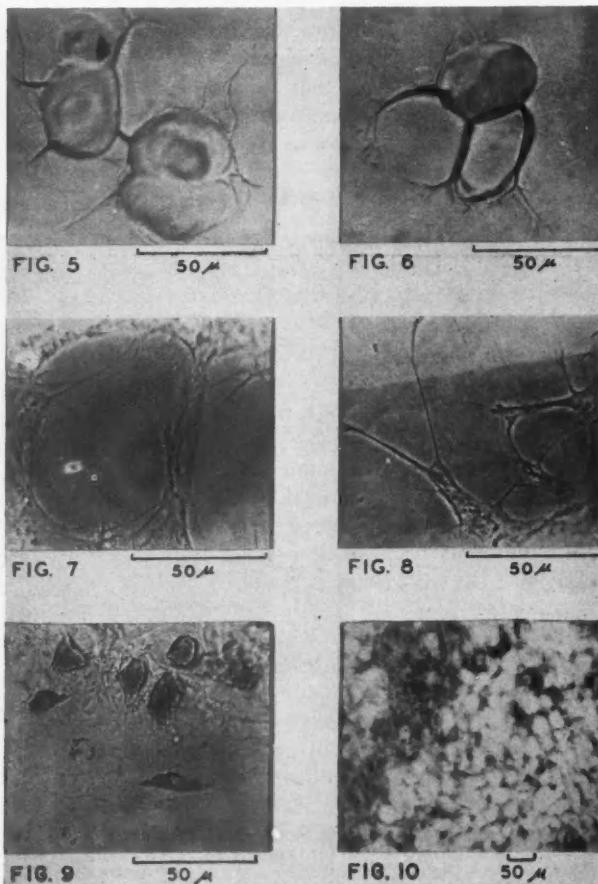
FIG. 5. Surface of a calcite crystal into which *Cliona celata* had begun boring. Three cavities left by removal of calcite fragments can be seen, and an adjacent pattern of lines etched into the crystal. Transmitted light.

FIG. 6. Another surface view of crystal shown in Fig. 5. One fragment has been removed, and outlines of more have been etched into the calcite.

FIG. 7. Living sponge cells left on a cover glass after removal of culture of *Cliona celata*, photographed by phase contrast. Note the cytoplasmic films and filaments forming figures resembling those etched into calcite (Figs. 5, 6). A few bacteria are scattered over the exposed glass. The three light spots are artifacts.

FIG. 8. Another view of living sponge cells on a cover glass from which a culture of *Cliona celata* has been removed. Cytoplasmic films and fine pseudopods are visible.

FIG. 9. Edge of living culture of *Cliona celata* on cover glass. Neutral red stain; transmitted light. The nucleus of a cell of the type which produces films and threads of cytoplasm is surrounded by a circle of stained granules, but its cytoplasm is scarcely visible. The numerous very granular amoebocytes do not appear to be directly involved in boring.

FIG. 10. Paraffin section of culture of *Cliona celata* boring in calcite, cut parallel to crystal face. Stained with iron haematoxylin and eosin. The numerous cavities contained calcite fragments which have been destroyed by fixation.

These began to bore as soon as metamorphosis was complete, before any spicules had formed, thus confirming Nassanov's (10) statement.

Fragments removed by *Cliona* from the shells of oysters (Fig. 1), from *Venus mercenaria* (Fig. 2), and from crystals of calcite (Fig. 3) are identical in shape and size. Each is bounded by several curved faces, some convex and some concave, which meet in sharp edges. Fragments of this shape are not produced when a shell is broken by pressure or hammering; and if a large crystal of calcite is reduced to powder by mechanical means, it usually breaks cleanly along planes parallel with the crystal faces, so that the smallest fragments are still typical crystals, bounded by plane surfaces and straight edges.

Most calcite crystals are marred by scratches and flaws; *Cliona* showed no inclination to attack these weak points, but began its borings anywhere on the surface of the crystal. Scratches made on shells and crystals with a needle had no influence on the location of borings.

To discover whether boring sponges dissolve calcium carbonate, I filled each of eight finger bowls with slightly more than 100 ml. of filtered sea water. In each of the four "test" dishes I placed a clean cover glass and a calcite crystal bearing an actively boring sponge; in the four control dishes I placed a clean calcite crystal, and a cover glass bearing a sponge. The dishes were covered with watch glasses, and set aside for 3 days. At the end of that time, I filtered the water from each dish and analyzed it for its calcium content.

The results of these analyses varied from 332 to 346 mg. calcium/liter; test dishes gave results both higher and lower than control dishes, but the differences between tests and controls were not generally larger than those between duplicate analyses on the same dish. During the period of this experiment, two or three of the more active sponges discharged 10 to 15 mg. of calcite fragments. Had they dissolved this amount of calcite, the calcium content of the water would have risen by more than 50 mg./liter.

When oyster shells are dissolved in hydrochloric acid, sheets of an organic substance, conchiolin, which often separate pearly layers of the shell, are left behind. These give a positive reaction with certain tests for proteins (Medcof (7)). When spongy shells were dissolved in acid the sheets of conchiolin were found to be pierced by holes bounded by partially detached flakes (Fig. 4). These were similar in shape to cross sections of shell fragments detached by the sponge (Figs. 1 and 2). Wax casts of excavations in oyster shells showed a definite layered construction, most of the tunnels lying parallel to the faces of the shells, with only a few perpendicular tunnels passing through the conchiolin sheets—just as a coal mine might have many layers of horizontal galleries in the coal, with only a few vertical shafts passing through the intervening strata of slate. Shells of living mussels were never found to be spongy until the horny periostracum had been abraded near the umbo; sponges planted on the inner surfaces of empty mussel shells attacked them readily, but those planted on the outer surface failed to pierce the

periostracum. All these observations suggest that *Cliona* does not pierce conchiolin readily. Nevertheless, in nature, boring sponges often cause severe damage to the hinge ligaments of oysters, which are of conchiolin.

The surface of a calcite crystal into which a sponge had begun to bore showed a pattern of fine curved lines, outlining areas similar in shape to those surrounding holes through conchiolin. Similar lines, not yet forming closed figures, branched from these (Figs. 5 and 6). The areas outlined by such lines were of the same shapes and dimensions as the calcite particles which the sponge disgorged. The lines were etched into the surface of the calcite, and could not be removed by polishing with fine abrasives ("Bon Ami"). No such lines were found on cover glasses on which sponges had been reared to the same age, nor were they ever found on sponge-free calcite crystals, even though these were kept in dishes with sponge cultures and became covered with the same microorganisms that surrounded the sponges.

With the phase contrast microscope, the cells left on a cover glass after a sponge had been gently wiped off showed a remarkable network of thread-like interconnections and pseudopodia, often 50 μ or more long. These surrounded areas reminiscent in shape of the calcite particles excavated by the sponges, or of the lines etched on calcite crystals. Other cells showed broad filmy expansions, only two or three microns thick (Figs. 7 and 8). These structures were hard to see with the ordinary microscope, and difficult to fix and stain. Similar pseudopodial extensions existed at the edges of reconstitution cultures, where they could be seen by ordinary microscopy; but the cytoplasm of the responsible cells was clear and transparent, and attention was distracted from it by another type of amoebocyte with a granular cytoplasm. Neutral red staining increased the difficulty of observation, as a mere ring of granules around the nucleus stained in the pseudopodial cells, while the other amoebocytes were filled with deeply staining particles (Fig. 9).

When a sponge culture on calcite was illuminated from below on the stage of the dissecting microscope, particles of calcite could clearly be seen moving with the exhalent streams through the canals at a speed of five or six centimeters per second to the oscula, where they were discharged. These particles rapidly accumulated in gleaming-white piles near the oscula, and sometimes completely surrounded them like a cone of volcanic debris. Within the parenchyma of the sponge could be seen masses of fragments, which moved perceptibly only in the course of several hours. Each of these particles was surrounded by cells, and a section through the region occupied by such a mass before acid fixation presents the appearance of foam, each cavity the size and shape of a calcite fragment (Fig. 10). The masses moved slowly toward nearby excurrent canals, into which the particles were discharged and carried away.

Discussion

In this investigation, I cannot claim to have advanced much farther toward a solution of the problem than did Nassanov and Topsent. However, my

technique of producing small sponges by the reconstitution of fragments has been exceedingly useful to me, and should be useful to others who wish to pursue the problem further.

These small sponges on calcite crystals permit the observation of many activities which cannot easily be studied otherwise, and which have not been described before; for example, movement of calcite particles through the sponge tissues to the excurrent canals. This must involve complex movements of the cells surrounding the particles. However, the movement of massive particles by sponge tissues is not rare. In reconstitution cultures of *Cliona*, spicules up to 350 μ long are rearranged and distributed peripherally with their points radiating outward. A *Cliona* culture on glass becomes surrounded by a growth of diatoms and other organisms, but a zone one millimeter or more wide remains clear because of the constant back-and-forth motion of the edge of the sponge. According to van Weel (15), particles more than 80 μ in diameter may be phagocytized by the surface epithelia of sponges.

What I have contributed to an understanding of boring justifies Nassanov's and Topsent's rejection of the idea that *Cliona* bores with its spicules, or dissolves its way through shells and stones by the copious secretion of acids.

Chemical action of some sort is strongly indicated, however, by the inability of the sponge to bore through anything but calcium carbonate, with the puzzling exception of conchiolin; and my results suggest that *Cliona* does not penetrate conchiolin as readily as Topsent and others believed. Other substances of a shell- or limestone-like consistency are unaffected by it. The obvious inference is that an acid is involved; but this must be produced in minute quantities; otherwise it would have been detected by my indicator solutions, or would have produced a noticeable increase in the calcium content of the water in which my finger-bowl cultures were kept.

Old (11) stated that "analyses of sea water containing active boring sponges in otherwise clean shells . . . show a slight daily increase in dissolved calcium". He did not describe his experimental technique, and it seems possible that the increase in calcium which he observed may be due to the action of other organisms on the shells (it is almost impossible to obtain a spongy oyster shell which is "otherwise clean") or even to evaporation of water in his containers. I feel confident that in my experiments I would have detected a consistent increase in calcium of the order of 5 mg./liter. In other words, if the sponge dissolves any of the calcium it removes, it must be less than 10% of it.

On the other hand, my results indicate that a purely mechanical explanation of boring, such as that proposed by Letellier (6), must be excluded. Calcite crystals and shells do not produce similar fragments when broken mechanically, and calcite, at least, breaks into fragments very different from those removed by the sponge.

The striking resemblance in pattern between the lines etched into calcite and the cytoplasmic reticulum underlying the sponge suggests that an acid, or some other solvent, is secreted in minute quantities by the threads of

cytoplasm. This would seem to be the only way that pseudopodia and films of cytoplasm could insinuate themselves into the calcite to excavate particles of such consistent sizes and shapes regardless of differences in substratum. I might suggest an analogy with tunnelling through limestone, not by spraying acid on it from a hose, but by carving out blocks with acid dispensed from a pipette.

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PREFERRED TEMPERATURE OF RAINBOW TROUT (SALMO GAIRDNERI RICHARDSON) AND ITS UNUSUAL RELATIONSHIP TO ACCLIMATION TEMPERATURE[1]

E. T. GARSIDE AND J. S. TAIT

Abstract

The modal preferred temperatures of rainbow trout acclimated to 5° C., 10° C., 15° C., and 20° C. were determined photographically to be 16° C., 15° C., 13° C., and 11° C., respectively. The final preferendum was 13° C. The phenomenon of decreasing preferred temperature with increasing acclimation temperature has not been reported for any other species of fish.

Introduction

The purpose of this study was to determine the selected or preferred temperature of the rainbow trout, *Salmo gairdneri* Richardson, at various acclimation temperatures. The preferred temperature of fish generally has been found to be influenced by the temperature at which they are acclimated. In nearly all of the species for which the preferred temperature has been determined at several acclimation temperatures it bears a positive relationship to increasing acclimation temperature. For example this is true for goldfish (*Carassius auratus*) (Fry (3)), *Girella* (Doudoroff (2)), spring (*Oncorhynchus tshawytscha*) and pink (*O. gorbuscha*) salmon (Brett (1)), and carp (*Cyprinus carpio*) (Pitt *et al.* (4)). The point at which the preferred temperature equals the acclimation temperature is the final preferendum (Fry (3)).

Materials and Methods

The fish were reared at the laboratory from eggs supplied by the Provincial Fish Hatchery at Normandale, Ontario, in May 1955. They were incubated at approximately 9° C., and, after hatching, rearing was continued at this temperature. In September 1956 four groups of 11 fish ranging in size from 4 to 6 in. were acclimated at 5° C., 10° C., 15° C., and 20° C. respectively for a minimum of 1 month. The group acclimated at 20° C. was raised to that temperature gradually and the total acclimation period was 12 weeks.

The experiments were performed in a glass aquarium of the type illustrated in Brett (1) 36 by 36 by 20 in. with a shallow V-shaped bottom. One of the broad sides of the aquarium designated as the front was marked with horizontal lines to form 10 zones. A screen was placed at the bottom of the lowest zone to exclude the fish from the V-shaped bottom. A thermometer was placed horizontally at the right-hand front center of each zone. For a given experiment, the tank was filled initially with water at 2° C. or 3° C. To set up and maintain the temperature gradient warm water was circulated from top to bottom through a heat exchanger consisting of a rectangular

coil of six turns of aluminum tubing suspended in the aquarium. To maintain the low end of the gradient refrigerated brine was circulated through a set of parallel aluminum tubes at the bottom of the tank. When the gradient had been partly established, the fish were placed in the tank. They were there for 1 to 1½ hours before recording of positions was begun. During this time establishment of the gradient was completed, and the fish recovered sufficiently from the disturbances caused by their transfer to swim freely around the tank in an apparently normal manner.

The gradient ranged from 4° C. to 24° C. through a distance of 33 in.: the temperature change was approximately two Centigrade degrees for each 3 in.

In previous experiments on temperature selection of fish in a vertical gradient the manner of recording positions of the fish was that of direct observation (Brett (1), Pitt *et al.* (4), Fry (3)). In this method of observation there are possibly errors resulting from the movements of the fish. These errors would be increased if the activity of the fish increased at higher acclimation temperatures. To remove this source of error in the present experiment the positions of the fish were recorded photographically with a 35 mm. camera on panchromatic film with an ASA rating of 160 tungsten. With this film, flash bulbs, which might have had a disturbing effect on the fish, were unnecessary. The room in which the experiments were conducted was illuminated by daylight through a skylight and windows on the north side of the room. Additional light was projected through one end of the tank from a 150-w. flood lamp placed 5 ft. from it. This light removed reflections of nearby objects from the front and back glass plates. There was no detectable vertical light gradient. The photographs were taken at 1 minute intervals with a 5 minute pause after every tenth exposure. The temperature in each zone was recorded immediately before and after each group of 10 photographs. The mean of the two readings for each zone was taken as the temperature of the zone for the 10 photographs. The number of photographs taken for each experiment varied from 74 to 79.

A 35 mm. projector equipped with a carriage for strip film was used to observe the developed films. No difficulty was experienced in reading the negatives; the essential items, the fish and the zone lines on the glass front, were clearly visible.

The effect of parallax on the accuracy of recording the positions of fish was not serious except in the two zones, No. 2 and No. 9, adjacent to the top and bottom zones of the tank. This effect was practically eliminated in the reading of the films. For example, a fish appearing in a photograph to be in the bottom of zone 9 was recorded as being in zone 10 if it was near the back of the tank or in zone 9 if near the front.

Results

The frequency distribution of the fish in the temperature gradient was determined for each acclimation temperature. Table I gives the percentage frequency distribution of the fish. The modal and the mean preferred

temperatures are highest for the fish acclimated to 5° C. and are progressively lower for the fish acclimated to the higher temperatures.   On the basis of the ordinarily accepted tests the mean at each acclimation temperature is significantly different from the rest.   Thus there is no doubt as to the statistical significance of the trend.   In the experiments for 5° C. and 20° C. acclimation repeated at a later time, the values for the mean preferred temperatures differ by a small but statistically significant amount from the first determinations.   However, they are significantly different from the rest of the means and thus confirm the trend of lower preferred temperatures at higher acclimation temperatures.

In Fig. 1 the intersection of the curve of preferred temperatures with the 45° construction line drawn through the origin gives the value, 13° C., as the final preferendum for rainbow trout in these experiments.

TABLE I

THE PERCENTAGE FREQUENCY DISTRIBUTIONS IN A TEMPERATURE GRADIENT
FOR RAINBOW TROUT ACCLIMATED TO VARIOUS TEMPERATURES

| Temp.,° C. | Acclimation temperatures | | | | | |
| | 5° C. | | 10° C. | 15° C. | 20° C. | |
	Dec. 8	Aug. 29	Mar. 16	Oct. 28	Dec. 2	Sept. 13
25						
24			0.2			
23	0.5		0			
22	2.0		0	0.4		
21	8.9		0	1.2		
20	3.2		0.2	0		
19	3.9	5.8	0	2.4		0.6
18	3.6	6.3	1.3	3.0		2.6
17	14.1	10.0	2.0	3.7		3.6
16	14.9	19.9	7.6	10.4	0.2	4.1
15	14.2	25.1	46.1	7.6	0.6	9.4
14	13.1	10.7	17.4	12.2	0.4	9.0
13	6.8	6.9	10.5	24.0	14.3	16.0
12	3.2	4.8	1.8	22.5	35.4	21.4
11	6.1	9.1	6.5	7.1	42.8	16.5
10	4.8	2.4	2.2	0	5.3	5.3
9	0		1.1	1.4	1.0	8.8
8	0.7		2.9	1.4		0.2
7			0	2.8		1.7
6			0.2			0
5						0.4
4						0.4
Total observations	559	461	551	565	495	532
No. of fish	8	7	8	8	7	7
Preferenda						
Mode	16	15	15	13	11	12
Mean	15.7	14.9	14.1	13.5	11.6	12.5
S.D.	3.1	2.2	2.0	2.5	0.9	2.4

coil of six turns of aluminum tubing suspended in the aquarium.   To maintain
the low end of the gradient refrigerated brine was circulated through a set
of parallel aluminum tubes at the bottom of the tank.   When the gradient
had been partly established, the fish were placed in the tank.   They were
there for 1 to $1\frac{1}{2}$ hours before recording of positions was begun.   During this
time establishment of the gradient was completed, and the fish recovered
sufficiently from the disturbances caused by their transfer to swim freely
around the tank in an apparently normal manner.

The gradient ranged from 4° C. to 24° C. through a distance of 33 in.:
the temperature change was approximately two Centigrade degrees for each
3 in.

In previous experiments on temperature selection of fish in a vertical
gradient the manner of recording positions of the fish was that of direct
observation (Brett (1), Pitt *et al.* (4), Fry (3)).   In this method of observation
there are possibly errors resulting from the movements of the fish.   These
errors would be increased if the activity of the fish increased at higher accli-
mation temperatures.   To remove this source of error in the present experi-
ment the positions of the fish were recorded photographically with a 35 mm.
camera on panchromatic film with an ASA rating of 160 tungsten.   With
this film, flash bulbs, which might have had a disturbing effect on the fish,
were unnecessary.   The room in which the experiments were conducted
was illuminated by daylight through a skylight and windows on the north
side of the room.   Additional light was projected through one end of the tank
from a 150-w. flood lamp placed 5 ft. from it.   This light removed reflections
of nearby objects from the front and back glass plates.   There was no
detectable vertical light gradient.   The photographs were taken at 1 minute
intervals with a 5 minute pause after every tenth exposure.   The temperature
in each zone was recorded immediately before and after each group of 10 photo-
graphs.   The mean of the two readings for each zone was taken as the
temperature of the zone for the 10 photographs.   The number of photographs
taken for each experiment varied from 74 to 79.

A 35 mm. projector equipped with a carriage for strip film was used to
observe the developed films.   No difficulty was experienced in reading the
negatives; the essential items, the fish and the zone lines on the glass front,
were clearly visible.

The effect of parallax on the accuracy of recording the positions of fish
was not serious except in the two zones, No. 2 and No. 9, adjacent to the
top and bottom zones of the tank.   This effect was practically eliminated
in the reading of the films.   For example, a fish appearing in a photograph
to be in the bottom of zone 9 was recorded as being in zone 10 if it was near
the back of the tank or in zone 9 if near the front.

Results

The frequency distribution of the fish in the temperature gradient was
determined for each acclimation temperature.   Table I gives the percentage
frequency distribution of the fish.   The modal and the mean preferred

temperatures are highest for the fish acclimated to 5° C. and are progressively lower for the fish acclimated to the higher temperatures.   On the basis of the ordinarily accepted tests the mean at each acclimation temperature is significantly different from the rest.   Thus there is no doubt as to the statistical significance of the trend.   In the experiments for 5° C. and 20° C. acclimation repeated at a later time, the values for the mean preferred temperatures differ by a small but statistically significant amount from the first determinations.   However, they are significantly different from the rest of the means and thus confirm the trend of lower preferred temperatures at higher acclimation temperatures.

In Fig. 1 the intersection of the curve of preferred temperatures with the 45° construction line drawn through the origin gives the value, 13° C., as the final preferendum for rainbow trout in these experiments.

TABLE I

THE PERCENTAGE FREQUENCY DISTRIBUTIONS IN A TEMPERATURE GRADIENT
FOR RAINBOW TROUT ACCLIMATED TO VARIOUS TEMPERATURES

Temp.,° C.	Acclimation temperatures					
	5° C.		10° C.	15° C.	20° C.	
	Dec. 8	Aug. 29	Mar. 16	Oct. 28	Dec. 2	Sept. 13
25						
24			0.2			
23	0.5		0			
22	2.0		0	0.4		
21	8.9		0	1.2		
20	3.2		0.2	0		
19	3.9	5.8	0	2.4		0.6
18	3.6	6.3	1.3	3.0		2.6
17	14.1	10.0	2.0	3.7		3.6
16	14.9	19.9	7.6	10.4	0.2	4.1
15	14.2	25.1	46.1	7.6	0.6	9.4
14	13.1	10.7	17.4	12.2	0.4	9.0
13	6.8	6.9	10.5	24.0	14.3	16.0
12	3.2	4.8	1.8	22.5	35.4	21.4
11	6.1	9.1	6.5	7.1	42.8	16.5
10	4.8	2.4	2.2	0	5.3	5.3
9	0		1.1	1.4	1.0	8.8
8	0.7		2.9	1.4		0.2
7			0	2.8		1.7
6			0.2			0
5						0.4
4						0.4
Total observations	559	461	551	565	495	532
No. of fish	8	7	8	8	7	7
Preferenda						
Mode	16	15	15	13	11	12
Mean	15.7	14.9	14.1	13.5	11.6	12.5
S.D.	3.1	2.2	2.0	2.5	0.9	2.4

FIG. 1. Relation of the preferred temperature to acclimation temperature in rainbow trout.

Discussion

Contrary to the observed trend in other species of fish, the preferred temperature of rainbow trout is 3° C. to 5° C. lower when they are acclimated to 20° C. than when they are acclimated to 5° C. We know of no other species that have been found to exhibit this phenomenon. However, there are two species of salmon that deviate from the general pattern and perhaps suggest the condition found in rainbow trout. Chum salmon (*Oncorhynchus keta*) selected the same temperature at 10° C., 15° C., and 20° C. acclimation temperatures (Brett (1)). Sockeye salmon (*O. nerka*) show a rise in preferred temperature from 10.5° C. at 5° C. acclimation to 14.5° C. at 15° C. acclimation, falling to 13.5° C. at 20° C. acclimation.

No attempt to explain this phenomenon is offered here, but it may be pointed out that the observed changes in preferred temperature are not likely to be seasonal changes such as Sullivan and Fisher (5) have reported for speckled trout (*Salvelinus fontinalis*). In the first series of experiments those for fish acclimated to 5° C. and 20° C. were performed in December and the repeated experiments for these acclimation temperatures in late August and early September with essentially the same results.

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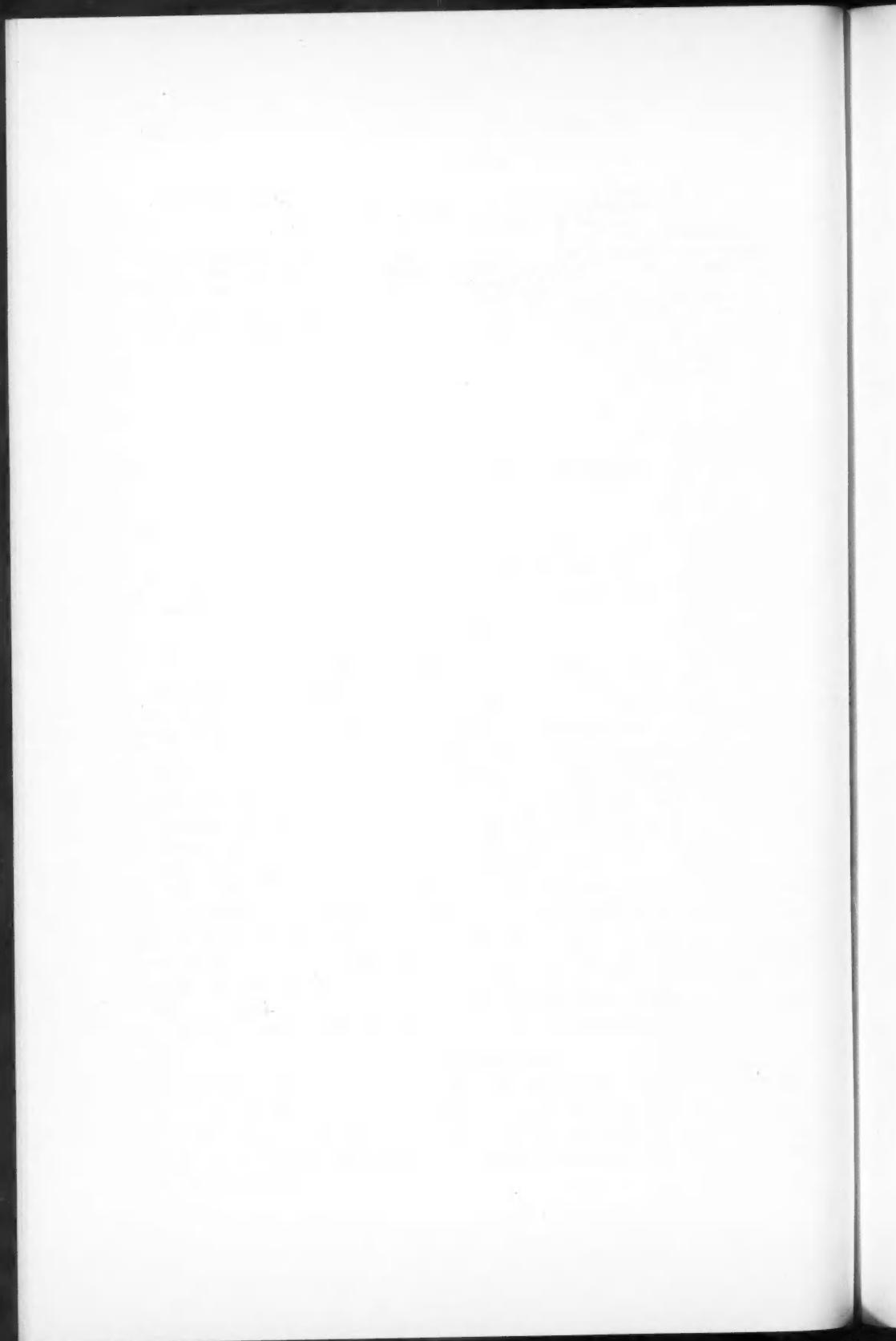
MORPHOLOGY OF DITYLENCHUS DESTRUCTOR THORNE, 1945 (NEMATODA : TYLENCHIDAE), FROM A PURE CULTURE, WITH SPECIAL REFERENCE TO REPRODUCTIVE SYSTEMS AND ESOPHAGEAL GLANDS[1]

LIANG-YU WU[2]

Abstract

Specimens of a pure line population of *Ditylenchus destructor* were studied. The female reproductive system is well differentiated into the ovary, oviduct, and uterus. The last is further differentiated into the seminal receptacle, quadricolumella, and uterus proper. There is a postuterine pouch, and this, with the vulva, is also described. The functional regions of the male reproductive system are distinct: the testis, vas deferens, seminal vesicle, and ejaculatory duct.

The posterior ends of the two subventral esophageal glands extend caudad to about the same level, each with a small nucleus. The large dorsal gland, with a conspicuous, larger nucleus, extends behind the esophago-intestinal junction. The esophageal tube leaves the esophagus on the inner surface of the dorsal gland, just behind the subventral glands, where it joins the intestine. A pair of very small cells guard the entrance of the esophagus into the intestine.

Introduction

Plant nematology is a comparatively young but rapidly growing branch of helminthology. As a large number of new species will undoubtedly be described in the future, some new approaches to the study of these nematodes seem necessary. Comparison of the literature on platyhelminths with that on nematodes indicates that detailed studies on the internal morphology of the former have contributed more to the solution of many problems in taxonomy than has been the case with nematodes. This may be due to the fact that the detailed internal morphological study of nematodes is more difficult, both in living and in preserved specimens after clearing, because the internal organs are not usually so well displayed as in the other forms. The organs are rather crowded together in the body cavity; and one structure may obscure another, particularly the intestine, the cellular walls of which may have numerous globules and granules. This study deals with the detailed morphology of the reproductive system and the esophageal glands of *Ditylenchus destructor* Thorne. In the past these two systems have been found to have some taxonomic value and more detailed information on them may prove useful. The features reported are evident from study and dissection of living specimens as well as mounted material.

Materials and Methods

The nematodes used were from a culture that has been maintained at the Ottawa laboratory for some time. This culture was propagated from a single gravid female isolated from a naturally infested potato tuber. The

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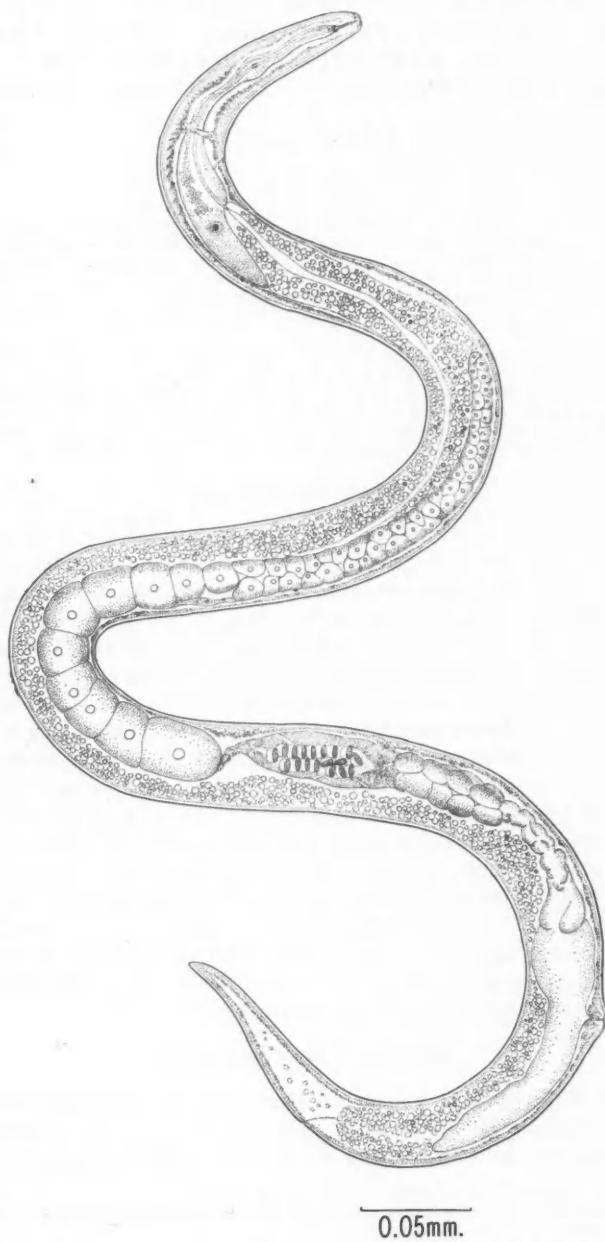
FIG. 1. *Ditylenchus destructor*.   Typical shape of living nematode.

studies were made chiefly with living and freshly dissected specimens in saline and stained with methylene blue.   The method of dissecting was the same as that used for studying *Trichinella spiralis* (Owen, 1835) Railliet, 1895 (3), except that the cuticle of these plant nematodes appears to be rather tough, so that No. 1 insect pins were used for dissecting.   These pins were sharpened on an oilstone into fine needles or into blades for cutting.   Only a single puncture or cut was made near the structure to be examined, and the pressure within the body cavity immediately expelled to the exterior the parts to be examined.   Living specimens anesthetized with dichlorodiethyl ether were also used for observation.   Measurements were made from specimens killed with heat, fixed in formalin–triethanolamine solution, stained with cotton blue, and mounted in lactophenol.

General Description

After being removed from the potato tuber and kept in water, these nematodes often assume a spirochaetal outline (Fig. 1).   The wall of the intestine is full of globules throughout its length.   This gives a fairly dark appearance to the female when it is alive.   In the male, the posterior half of the body cavity is chiefly occupied by the colorless reproductive system and the intestine is not massive in this region.   Therefore, male specimens can be easily recognized by their having a comparatively colorless posterior half, in addition to their slender, shorter body and sex organs.

Measurements of 80 female specimens (except where otherwise indicated) gave the following ranges: length, 1.007 to 1.396 mm.; width, 29.7 to 43.2 μ; distance between anterior end and valve of median bulb, 0.049 to 0.059 mm. (64 specimens); distance between valve of median bulb and esophago-intestinal junction, 0.064 to 0.088 mm. (64 specimens); distance between anterior end and tip of ovary, 0.098 to 0.440 mm.; distance between anterior end and vulva, 0.833 to 1.156 mm.; distance between tip of ovary and vulva, 0.430 to 0.940 mm.; distance between vulva and anus, 0.110 to 0.173 mm.; tail, 0.068 to 0.098 mm.; a = 26.27–39.40, with 71% between 29 and 34; b = 7.81–10.60, with 82% between 8 and 10 (64 specimens); and c = 11.67–17.21, with 76% between 13 and 16; $V = {}^{38.40-76.89}76.60-83.61^{6.30-10.37}$, with 84% between 80 and 83; distance between vulva and anus divided by tail length, 1.30–2.00; total length divided by length from vulva to anus, 7.19–9.69; and length from vulva to anus, 9.16–13.90% of total length.

Measurements of 40 male specimens gave the following ranges: length, 0.929 to 1.232 mm.; width, 26.1 to 33.3 μ; distance between anterior end and valve of median esophageal bulb, 0.050 to 0.062 mm.; distance between valve of median esophageal bulb and esophago-intestinal junction, 0.069 to 0.086 mm.; distance between anterior end and tip of testis, 0.205 to 0.500 mm.; distance between anterior end and anus, 0.900 to 1.145 mm.; distance between tip of testis and anus 0.500 to 0.805 mm.; a = 32.10–41.25 with 75% between 34 and 38; b = 6.30–9.40 with 85% between 7 and 8; c = 12.69–16.29 with 75% between 13 and 15; T = 51.38–73.15 with 72% between 57 and 67; and distance from anus to anterior end, 92.11–93.86% of total length.
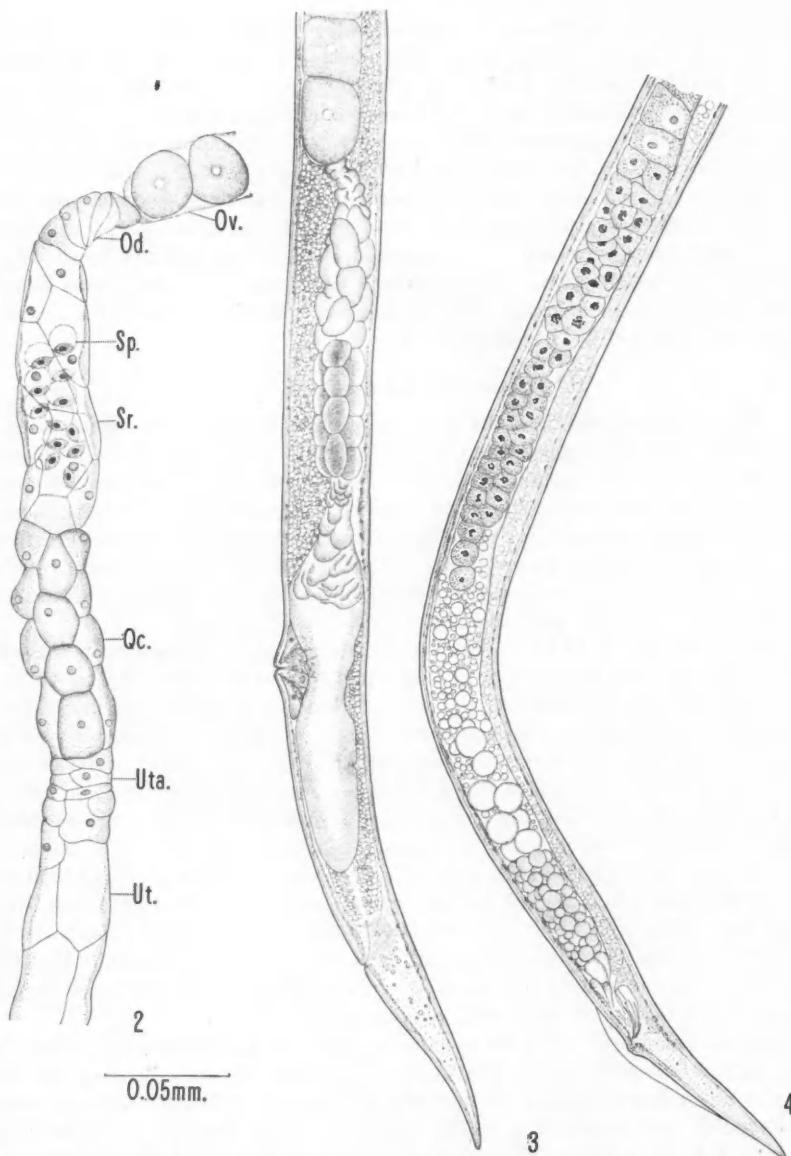
FIGS. 2–4. *Ditylenchus destructor*. 2. Portion of female reproductive system from dissected specimen in saline.   3. Posterior end of female.   4. Posterior end of male (only two spermatocytes are shown).

ABBREVIATIONS: *It*, intestine; *Ob*, median esophageal bulb; *Od*, oviduct; *Ov*, ovary; *Qc*, quadricolumella; *Sp*, spermatozoon; *Sr*, seminal receptacle; *Ut*, uterus; *Uta*, anterior area of uterus.
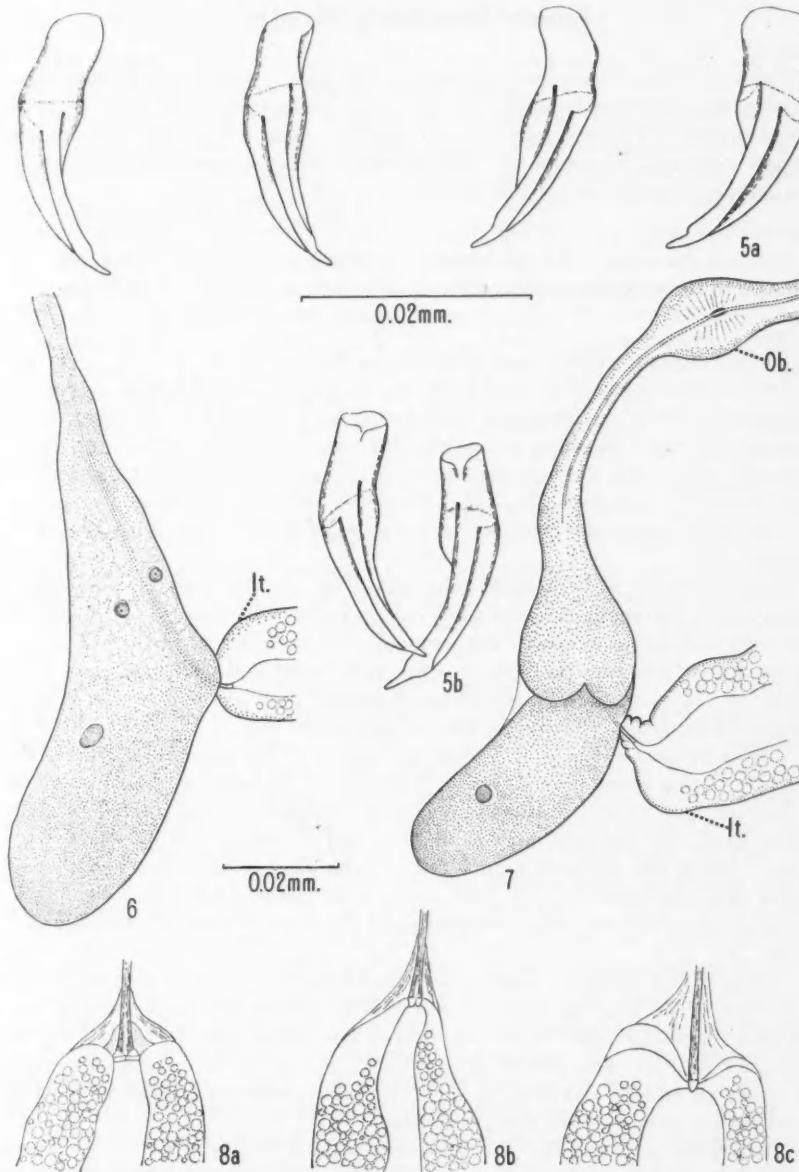
FIGS. 5-8. Spicules. *a*, a pair (lateral and ventral views) from a specimen dorsoventrally compressed. *b*, spicules from different specimens, laterally compressed. 6-7. Esophageal glands and esophago-intestinal junction, dissected from living specimens (drawings made with camera lucida). 8*a-c*. Diagrams showing "guard cells" at esophago-intestinal junction.

Female Reproductive System

Ovary

The ovary (Fig. 1) varies considerably in length.   Its tip usually lies some distance behind the esophagus.   In some specimens it extends to the level of the posterior end of the esophagus or further.   The developing oöcytes are usually arranged in two rows.   The more mature oöcytes are in a single row at the proximal end of the ovary.

Oviduct

Between the ovary and the uterus there is a short oviduct (Fig. 2).   It appears to be composed of two rows of cells, four to six cells in each row.

Uterus

Three different regions were observed in the uterus.

Seminal receptacle.—The distal end of the uterus is modified as a seminal receptacle.   In an undistended condition (Fig. 3) its wall is composed of a number of large cells, more or less glandlike in appearance.   However, these cells are very elastic and can be stretched to a considerable extent to form a comparatively thin wall (Fig. 2).   Therefore, when the seminal receptacle is filled with spermatozoa or during the passage of an ovum, it appears as a dilated tube.

Quadricolumella.—The quadricolumella (Fig. 3, the region immediately proximad to the seminal receptacle) consists of 16 cells (four rows of 4) which are very similar to those of the seminal receptacle when the latter is not distended.   However, they are coarsely granulated and darker in color than those of the receptacle.   Like them, however, they are elastic and can be greatly stretched to provide for the passage of the ova.

Uterus proper.—This is the proximal region of the uterus and two areas may be distinguished (Fig. 2, *Uta*, *Ut*; Fig. 3).   The anterior area is a slender structure, and its wall consists of a number of small but thick cells.   On the other hand, the posterior area is slightly oval to oblong and its wall is very thin.   When the uterus is empty the anterior part tends to telescope somewhat into the posterior part and appears as folds.   Sometimes the uterus may be stretched to a considerable extent to accommodate several eggs at a time.

Vulva.—The vulva is a fairly prominent structure with the lips somewhat elevated.   The opening is a transverse slit.   From the lateral view a pair of cells (vaginal glands?) were clearly seen associated with the vulva (Fig. 3). Another pair of cells, which are probably muscle cells, were seen pressed between these cells and the vagina.   The muscle cells are slightly granulated and deeper in color than the gland cells.

Postuterine pouch.—The shape of the uterine pouch (Figs. 1, 3) varies with age.   In young females it is a slender sac, whereas in older specimens the diameter is much greater.   The length varies considerably, in mature specimens from 0.074 to 0.128 mm. (in specimens 1.163 and 1.396 mm. long, respectively).   Very often a few male sex cells were seen in the pouch.   A cell was occasionally seen at the tip of the sac (not shown in the figure).

Male Reproductive System

This system may be divided into four functional regions (Fig. 4), although no clear demarcations between them were seen.

Testis.—The testis varies considerably in length. The anterior tip may reach to or beyond the esophago-intestinal junction but is usually some distance behind this point. The developing spermatocytes are usually arranged in two or more rows, but these are usually fewer at the proximal and distal ends. In less than 10% of the specimens examined, however, the spermatocytes were arranged in a single row.

Vas deferens.—Although there is no sharp demarcation separating this region from the testis, it has its functional entity. In this region, multiple rows of small cells are seen; this indicates that the spermatocytes undergo cell divisions. In the cytoplasm there are numerous rod-shaped structures. In the central area of these cells the chromatin masses gradually decrease in number, but increase in size, and finally assume the form of lobed nuclei. Some further changes appear to occur in the last portion of the vas deferens.

Seminal vesicle.—The posterior, dilated portion of the male genital tube functions as a seminal vesicle, because in this region there is a large accumulation of male sex cells that are finely granulated. Their sizes vary.

Ejaculatory duct.—The seminal vesicle is a dilated tube and as it ends a short distance from the cloaca the ejaculatory duct is probably represented by the intervening region, although this matter has not been definitely determined.

Spicules.—The two spicules are very similar. Each has a broad basal half and a narrow distal half in gross appearance (Fig. 5), and appears to have its edges rolled entad. Near its tip, it narrows abruptly to a fingerlike apex. The basal half of a spicule appears to be slightly more sclerotized than most of the distal region, giving the appearance of a ringlike structure crossing the basal third. There are two long, denser strips leading from the points where the reduction at the tip takes place. When both spicules are viewed from the ventral side (Fig. 5*a*), the strips toward the concave side and nearest the median line are usually the longer. These two rodlike strips appear to be two persisting structures, but they are also somewhat like the edges of a spoon which are apparently thickened and rolled inward.

Esophageal glands and esophago-intestinal junction.—The esophageal glands, especially the dorsal gland, are fairly large structures that give a club-shaped appearance to the posterior end of the esophagus (Fig. 1). The dorsal gland extends backwards so that part of it lies over the anterior end of the intestine. The nucleus of the dorsal gland is large and prominent.

From a dissected specimen, the three esophageal glands have the appearance of a single structure (Fig. 6). However, after having been in saline for some time, the two subventral glands appear to be vacuolated, and therefore stain more lightly, and the nuclei are smaller. The dorsal gland is much larger than the others and oblong in outline. In one specimen, a thin membrane was observed at the angle between one subventral and the dorsal gland

(Fig. 7).   This membrane may be the wall of the esophagus and be greatly distended to enclose the three glands.

The esophageal tube leaves the dorsal gland on one side just behind the subventral glands.   There is a clear area, without globules, at the anterior tip and on one side of the intestine.   Therefore the junction is located fairly easily in live and even in preserved specimens.   A pair of very small, flattened cells were seen guarding the entrance to the intestine (Fig. 8a).   The wall of the esophageal tube dilates slightly as it approaches the intestine.   Just above these two "guard cells" there are a pair of convex–conoid structures that may be muscular attachments or parts of the enlarged, sclerotized wall of the esophageal tube.   In another specimen, the two "guard cells" had a cuboidal shape (Fig. 8b), whereas in another specimen these two cells formed a convex–conoid structure (Fig. 8c) and were slightly projected into the lumen of the intestine.

Discussion

This study indicates that the female reproductive system of *D. destructor* is well differentiated, all the functional regions being well marked off from each other.   The term *quadricolumella* is tentatively assigned to the structure having four rows of cells (four cells to a row in this species) situated behind the seminal receptacle.   Although the function of these cells is not yet clear, their granulated appearance suggests that they have some secretory function. Steiner (1) indicated in his figure for *Ditylenchus askenasyi* a much greater number of cells for this structure.   As the number in each row is constant in *D. destructor*, this structure may have some taxonomic value.   The seminal receptacle and the quadricolumella are here considered as modifications of parts of the uterus, because when they are stretched the cells become flattened and, therefore, more or less of the squamous type.   When the pressure on the wall of the seminal receptacle is released, as in a dissected specimen, the spermatozoa appear as spherical structures filled with clear fluid, and on one side there is an oval mass of protoplasm with a very conspicuous nucleus. The male sex cells found in the seminal vesicle of the male are different from the spermatozoa seen in the seminal receptacle.   This may indicate that development of the male elements continues after insemination.

Thorne (2) described a very small valvular apparatus at the esophago-intestinal junction.   As evidence here shows, this valvular apparatus consists of two "guard cells".

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HELMINTH PARASITES OF REPTILES, BIRDS, AND MAMMALS OF EGYPT

V. AVIAN CESTODES[1]

June Mahon[2]

Abstract

Twenty-nine species of cestodes are recorded from birds in Egypt, including two new species—*Raillietina* (*Fuhrmannetta*) *malakartis* (Davaineinae) and *Neyraia parva* (Paruterininae). There are several new host records.

Introduction

These cestodes form a part of a collection of helminths from a parasite survey of vertebrates made in Egypt by Dr. Robert E. Kuntz, Parasitology Department, Research Unit No. 3, Cairo, Egypt, from 1948 to 1953. It was indeed a pleasure to work with such excellent material.

The helminth fauna of Egypt has received a certain amount of attention, notably by A. Looss, the tapeworms of whose collection were largely described by F. J. Meggitt (26, 27, 28, 29). Since that time the emphasis has been mainly on parasites of medical and veterinary importance.

The avian cestodes only of the collection are the subject of this paper. Two new species are described and there are several new host records. The species studied are listed in systematic order. All drawings were made with the aid of a camera lucida. The host names used in the text, whether taken from the literature or from the collection, have been revised according to J. L. Peters (35) and Nicoll (32).

The writer would like to acknowledge her indebtedness to Professor T. W. M. Cameron, Director of the Institute of Parasitology, Macdonald College, for his kindly interest in the preparation of this paper.

The slide preparations of the material discussed will be deposited in the U.S. National Museum.

List of Cestodes

TETRABOTHRIDEA BAER, 1954

TETRABOTHRIIDAE BRAUN, 1900

Tetrabothrius sp.

CYCLOPHYLLIDEA BRAUN, 1900

DAVAINEIDAE FUHRMANN, 1907

Davaineinae Braun, 1900

Cotugnia polycantha Fuhrmann, 1909
Raillietina (Raillietina) fuhrmanni idiogenoides (Baer, 1933)
Raillietina (Raillietina) galeritae (Skrjabin, 1915)
Raillietina (Fuhrmannetta) malakartis n. sp.

ANOPLOCEPHALIDAE FUHRMANN, 1907

Anoplocephalinae Fuhrmann, 1907

Aporina delafondi (Railliet, 1892)

DILEPIDIDAE FUHRMANN, 1907

Dilepidinae Fuhrmann, 1907

Amoebotaenia brevicollis Fuhrmann, 1907
Angularella beema (Clerc, 1906)
Angularella sp.
Anomotaenia aegyptica (Krabbe, 1869)
Anomotaenia dehiscens (Krabbe, 1879)
Anomotaenia nymphea (Schrank, 1790)
Dilepis sp.
Lateriporus merops Woodland, 1928

Dipylidinae Stiles, 1896

Choanotaenia marchali (Mola, 1907)
Choanotaenia strigium Joyeux and Timon-David, 1934
Choanotaenia sp.

Paruterininae Fuhrmann, 1907

Anonchotaenia globata (v. Linstow, 1879)
Anonchotaenia sp.
Neyraia intricata (Krabbe, 1882)
Neyraia parva n. sp.

HYMENOLEPIDIDAE FUHRMANN, 1907

Hymenolepidinae Perrier, 1897

Diorchis longicirrosa Meggitt, 1927
Haploparaxis filum (Goeze, 1782)
Hymenolepis bilharzi (Krabbe, 1869)
Hymenolepis fringillarum (Rudolphi, 1809)
Hymenolepis pauciannulata Meggitt, 1927
Hymenolepis stylosa (Rudolphi, 1809)
Hymenolepis spp.

TAENIIDAE LUDWIG, 1886

Cladotaenia sp.

Tetrabothridea Baer, 1954

Tetrabothrius sp.

Host: Anas querquedula L. (Anseriformes) 2979; Egypt.

Two detached scoleces were recovered. In anterior view, the scolex is square in outline and has a diameter of 845 μ. The four suckers measure 310 to 324 μ across; the lateral appendices of the suckers are not well developed.

The only species recorded from the Anseriformes is *Tetrabothrius arcticus* v. Linstow, 1901 from *Somateria mollissima* L. from Spitzberg. Baer (3), on re-examining the type specimens, considers *T. arcticus* to be identical with *T. immerinus* (Abildgaard, 1790), a parasite typically from Gaviiformes and Colymbiformes, and points out the possibility of an error in the labelling of the host of v. Linstow's material. It is unfortunate that the present material is incomplete, but it would indicate that a *Tetrabothrius* species does occur in Anseriformes.

Cyclophyllidea Braun, 1900

Cotugnia polycantha Fuhrmann, 1909

Host: Streptopelia senegalensis senegalensis (L.) (Columbiformes) 1813, 1356; Giza. 1379, 1381, 1468; Qalubiya.

Examination of the descriptions of all species recorded from the Columbiformes revealed that these specimens agree most closely with *Cotugnia polycantha* originally described from *Streptopelia t. turtur* (L.) from Egypt. Fuhrmann's (9) measurements are given in parentheses for the purpose of comparison.

This species is represented by three complete specimens. The longest measures 32 mm. (35 mm.) and has a maximum breadth of 3 mm. (4 mm.) just anterior to the gravid segments.

The scolex, mounted in Canada balsam, has a diameter of 423 μ (450 μ). The suckers are small, diameter 87 μ (90 μ), but the rostellum is very wide, 219 μ (220 μ). The latter is armed with 300 to 320 (420) hammer-shaped hooks, arranged in a double crown. The hooks are 9 to 12 μ (10–12 μ) long.

Characteristic of this genus are the two sets of genitalia per segment. The testes are disposed in two lateral fields. In each field there are 17 to 25 aporal testes and 9 to 14 poral testes situated posteriorly to the genital ducts. Some testes may lie between the excretory vessels and the lateral margin of the segment. There is thus a total of 62 to 72 (100) testes in each segment.

The cirrus pouch is attenuated, non-muscular and narrow, measuring 204 to 255 μ (180 μ) by 36 μ. The uterus breaks down into capsules, each capsule enclosing one egg with a diameter of 44 μ.

The number of testes is rather smaller than in the previous records for *C. polycantha* and falls between the number given by Johri in 1934 (15), viz. 88 and the 45 to 55 given by Joyeux, Baer, and Martin in 1936 (19) for their variety *oligorchida*.

The genus is discussed and reviewed by Lopez-Neyra in 1950 (23).

Raillietina (*Raillietina*) *fuhrmanni idiogenoides* (Baer, 1933)

 Host: Streptopelia senegalensis senegalensis (L.) (Columbiformes) 1297;
   N. Cairo. 1356; Giza. 1813; Giza.

Baer (2) records this species from *Treron delandii grantii* (v. Someren)
from Rhodesia. The dimensions he gives are shown in parentheses.

The longest specimen is 90 mm. (50 mm.) in length and has a maximum
breadth of 1.2 mm. (0.68 mm.).

The genital pores are unilateral.

Mounted in gum chloral, the scolex measures 219 to 277 μ (250–260 μ) in
diameter. The suckers, armed with several rows of small spines, have a
diameter of 58 to 99 μ (80–100 μ). The rostellum is wide, diameter 124 to
197 μ, and is armed with about 230 (150–200) hooks arranged in a double
crown. The hooks have a length of 7 μ and 8 μ (5–8 μ).

The testes are few, 2 to 4 in the poral group and 6 to 11 aporally, totalling
9 to 14 (10–12) (Fig. 1). The cirrus pouch is weakly muscular and measures
145 to 164 μ (130–150 μ) in length and 69 to 55 μ (80 μ) in breadth.

The egg capsules contain from 6 to 8 (8) eggs each.

Thirty-three species of *Raillietina* (*R.*), characterized by unilateral genital
pores and several eggs per capsule, are recorded from the Columbiformes and
are listed by Hughes and Schultz in 1942 (13).

Raillietina (*Raillietina*) *galeritae* (Skrjabin, 1915)

 Host: Passer domesticus (L.) (Passeriformes) 1296; N. Cairo. 1319; Cairo.
   1407; Giza. 1423, 1 and 2; Giza.

The worms were fragmented, but the total length was estimated at 50 mm.
The maximum breadth is 1.75 mm.

The scolex, mounted in gum chloral, is 124 μ in diameter. The suckers are
apparently unarmed, but the spines have probably been lost. The rostellum,
diameter 51 μ, is armed with about 180 hammer-shaped hooks, each 9 μ in
length.

The genital pores are unilateral. The cirrus pouch measures 80 by 44 μ.
The testes number 20 to 26 and are arranged on each side and behind the female
organs (Fig. 2). The ovary is bilobed. There are 5 to 6 eggs in each paren-
chymatous capsule. The number of egg capsules per segment is very variable.

The species of this subgenus so far recorded from passeriform birds are
shown in Table I.

As seen from the table, the present material shows similarities to both
R. (*R.*) *sartica* var. *masiliensis* and to *R.* (*R.*) *galeritae*. The variety *masiliensis*,
typically found in sparrows, agrees with *R.* (*R.*) *sartica* in all respects save the
dimensions shown in the table.

The present material differs from *R.* (*R.*) *sartica* in lacking both vaginal and
cirrus pouch sphincters. Also the relative extent of the cirrus pouch in the
Egyptian material appears to be less than that indicated by Skrjabin's (39)
original drawings and measurements for *R.* (*R.*) *sartica*.

TABLE I

SPECIES OF *Raillietina (Raillietina)* FROM PASSERIFORMES

Species	sartica	sartica masiliensis	calyplomenae	galeriae	Present material
Author	Skrjabin, 1914 (39)	Joyeux and Timon-David, 1934 (21)	Baylis, 1926 (4)	Skrjabin, 1915 (40)	
Length	45 mm.	90 mm.	60-100 mm.	25 mm.	50 mm.
Breadth	2.5 mm.	2.0 mm.	0.9-1.15 mm.	1.5 mm.	1.75 mm.
Scolex	260 μ	190 μ	170-250 μ	180 μ	124 μ
Suckers	156 μ	70 μ	55-80 μ (armed)	74 μ	–
Rostellum	96 μ	80 μ	100-125 μ	62 μ	51 μ
No. of hooks	200	220-250	Very numerous	200	180
Length of hook	7.4-9 μ	9-10 μ	8 μ	10 μ	9 μ
Testes	30-35	25	5	35	20-26
Cirrus pouch	148×55 μ	110×50 μ	160×55 μ	75×38 μ	80×44 μ
Eggs/capsule	3-4	3-4	–	3-6	5-6
Host	*Corvus corone* L.	*Passer domesticus* (L.)	*Calyplomena whiteheadi*	*Galerida macrorhyncha* Frister.	*Passer domesticus* (L.)
Locality	Russian Turkestan	Marseille	Mt. Murud, Sarawak	Algiers	Egypt

Abbreviations used in table: Breadth: maximum breadth of strobila; cirrus pouch: length and maximum breadth of cirrus pouch; eggs: diameter of eggs; eggs/capsule: number of eggs per capsule; embryonic hooks: length of embryonic hooks; embryos: diameter of embryos; length: maximum total length of strobila; no.: number; rostellum: diameter (and length) of rostellum; scolex: diameter of scolex; suckers: diameter of suckers; testes: number of testes per segment.
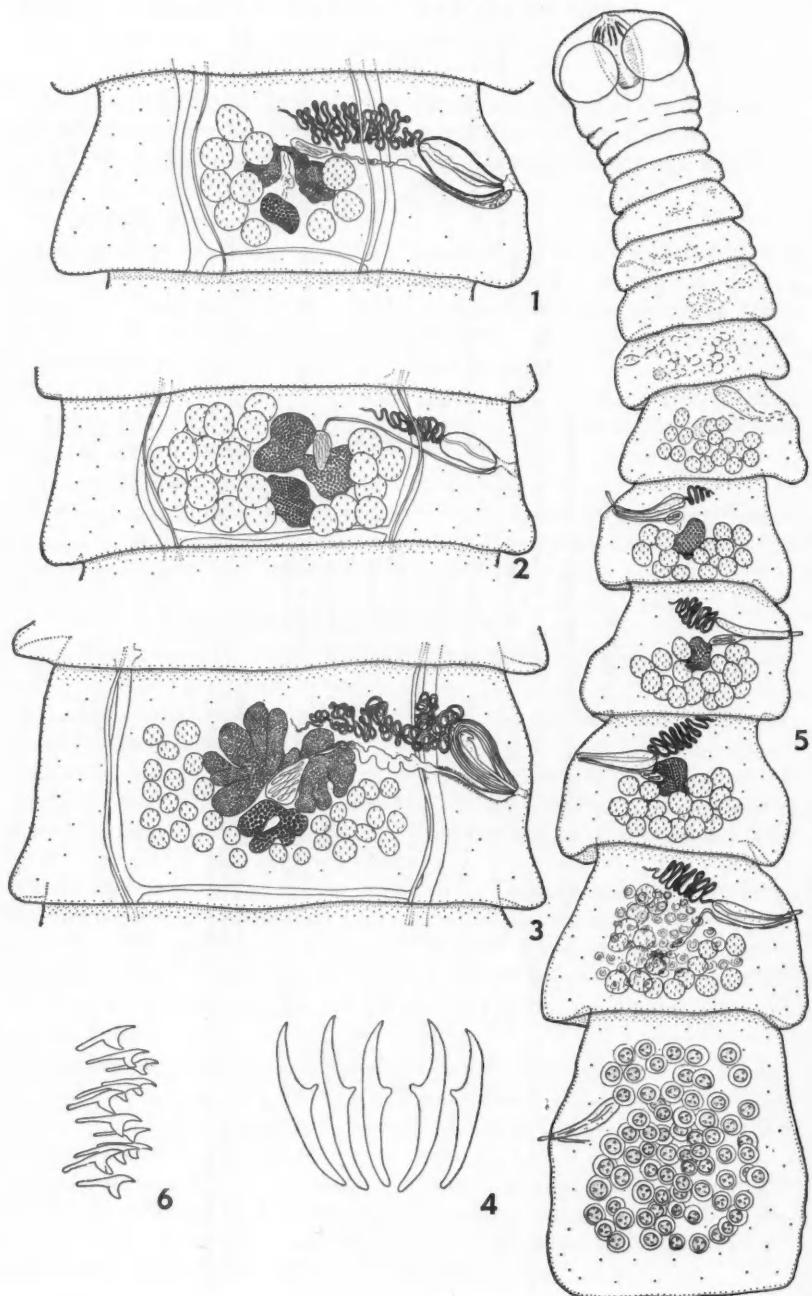
The general appearance of the mature segment resembles that described for *R. (R.) galeritae*. The dimensions of the cirrus pouch correspond, but there is a discrepancy in the number of testes, i.e., 35 in Skrjabin's (1915) (40) description and 20 to 26 in the present material.

For this group of worms, a difference of this order in the number of testes is not sufficient to distinguish the present material as a separate species, so it is ascribed to *R. (R.) galeritae*.

Raillietina (Fuhrmannetta) **malakartis** n.sp.

Host: Coturnix sp. (Galliformes) 1890; Giza. 1904; Minufiya.

There are several complete specimens; the material was well preserved.

The length of the type specimen is 90 mm. and the maximum breadth is 2 mm. The genital pores are irregularly alternating; all the segments are broader than long. Numerous calcareous bodies are seen in the cortical parenchyma.

The scolex, mounted in Canada balsam, has a diameter of 153 μ. The suckers, diameter 55 μ, are armed with several rows of small spines. The rostellum, diameter 105 μ, bears 150 to 160 hammer-shaped hooks arranged in a double crown. The hooks measure 9 to 9.7 μ in length.

The excretory system is of the normal type, with two pairs of longitudinal vessels, the dorsal ones being narrower than the ventral ones. The ventral vessels are joined by a posterior transverse commissure in each segment.

The testes number 24 to 33 (Fig. 3) and lie on either side of the female genital organs. There are 9 to 14 in the poral group and 13 to 22 aporally. The vas deferens is extremely convoluted; neither external nor internal seminal vesicle is present. The cirrus pouch is small, 146 to 160 μ by 65 to 73 μ, and does not reach the poral excretory vessels. The wall of the pouch, however, is thick and muscular and the lumen is small. The cirrus is sinuous and unarmed. The genital atrium is shallow and non-muscular.

The vagina opens at the genital atrium posterior to the cirrus pouch. The distal portion is narrow and appears to be enclosed within a sleeve of glandular cells. Together with the vas deferens, the vagina passes dorsal to both poral excretory vessels, its course then becoming sinuous and its lumen dilated and filled with spermatozoa. The vagina opens into a well-developed receptaculum seminis. The ovary, situated centrally in the segment, is deeply lobed and behind it lies the vitelline gland, which is also lobed.

The form of the uterus is indicated by the disposition of the eggs, which in gravid segments occupy the whole of the medulla between the excretory vessels. In the more posterior segments, parenchymatous capsules develop, each enclosing about 6 eggs.

FIG. 1. *Raillietina (R.) fuhrmanni idiogenoides*: dorsal view of mature segment.
FIG. 2. *R. (R.) galeritae*: dorsal view of mature segment.
FIG. 3. *R. (Fuhrmannetta) malakartis* n. sp.: dorsal view of mature segment.
FIG. 4. *Amoebotaenia brevicollis*: rostellar hooks.
FIG. 5. *A. brevicollis*: dorsal view of entire worm.
FIG. 6. *Angularella beema*: rostellar hooks.

TABLE II

SPECIES OF Raillietina (Fuhrmannetta) FROM GALLIFORMES

Species	pseudoechinobothrida	birmanica	laticanalis	globocaudata	pluriuncinata	malakartis
Author	Meggitt, 1926	Meggitt, 1926	(Skrjabin, 1914)	(Cohn, 1901)	(Crety, 1890)	n. sp.
Described by:	Southwell, 1930 (41)	Southwell, 1930 (41)	Joyeux & Baer, 1936 (17)	Joyeux & Baer, 1936 (17)	Joyeux & Baer, 1936 (17)	Present material
Length	80–90 mm.	8–10 mm.	110 mm.	19–20 mm.	105–120 mm.	90 mm.
Breadth	1.8 mm.	1–2 mm.	1.8–3.5 mm.	1.1 mm.	3 mm.	2 mm.
Scolex	—	—	200 μ	450–500 μ	313 μ	153 μ
Suckers	—	—	90 μ	100 μ	90 μ	85 μ
Rostellum	—	—	—	105 μ	—	105 μ
No. of hooks	200	300	150–220	Numerous	216	150–160
Hook length	8–12 μ	9–12 μ	16.5 μ	—	8–9 μ	9–9.7 μ
Testes	30–50	20–25	50–60	30	100–120	24–33
Cirrus pouch	Not reach exc. vessel	Extend past exc. vessel	150–170 ×50–70 μ	60 ×35 μ	—	146–160 ×65–73 μ
Eggs/capsule	3–4	Several	3–10	—	3–6	6
Host	"domestic fowl"	"domestic fowl"	Perdix sp.	Tetrao urogallus (L.)	Coturnix coturnix L. Caccabis petrosa Gm.	Coturnix sp.
Locality	Burma	Burma	Brazil	Europe	Europe	Egypt

NOTE: For abbreviations, see Table I.

The irregularly alternating genital pores and the egg capsules each enclosing several eggs place this species in the subgenus *Fuhrmannetta* Stiles and Orleman, 1926.

The characters of the species of this subgenus recorded from Galliformes are shown in Table II.

The very small cirrus pouch of *R*. (*F.*) *globocaudata* distinguishes it from the new species (i.e., 60μ cf. 146 to 160 μ). Both *R*. (*F.*) *pluriuncinata* and *laticanalis* are distinct because they possess a greater number of testes and because the latter has longer rostellar hooks. In *R*. (*F.*) *birmanica* the rostellar hooks are more numerous; the cirrus pouch is larger and extends median to the excretory vessels. It is altogether a much smaller worm. *R*. (*F.*) *malakartis* n. sp. is most closely related to *R*. (*F.*) *pseudoechinobothrida* Meggitt, 1926. It differs from the latter in the following respects: the number of rostellar hooks is less; there are fewer testes and the number of eggs per capsule is greater. From Meggitt's original description (25) and that of Southwell (41) it is impossible to compare other features of the anatomy, even the length of the cirrus pouch, so it is proposed to create a new species, *R*. (*F.*) *malakartis*, until such time as it can be compared with further material from the domestic fowl in Burma.

Aporina delafondi (Railliet, 1892)

Host: *Columba livia* Gm. (Columbiformes) 3023; Western Desert.

The longest worm measures 60 mm. and has a maximum breadth of 4 mm. The scolex has a diameter of 182 to 189 μ, and is provided with four suckers measuring 73 to 80 μ. The rostellum is lacking. The strobila does not narrow behind the head.

The genital pores are irregularly alternating, and the genital ducts pass dorsal to the excretory vessels. The posterior ventral transverse commissure is anastomosed.

The testes are disposed in two fields, on either side of the female glands, numbering 30 to 35 porally and 63 to 77 aporally, there being 93 to 114 in each segment. The cirrus pouch is small and does not extend to the poral excretory vessel. It has a length of 240 to 263 μ and a breadth of 44 to 47 μ. An internal seminal vesicle is present.

The female glands are situated slightly porally. The vagina passes posteriorly to the vas deferens and opens into a well-developed receptaculum seminis. The ovary is digitate, and behind it lies the compact vitelline gland. The uterus develops two, backwardly directed, diagonal prolongations, extending laterally to the excretory vessels but not passing them.

Fuhrmann, 1902, erected the genus to accommodate *Aporina alba* from a psittaciform from Brazil. In this form the testes surround the female organs, the genital pores are lacking, and the forwardly-directed uterine caecae lie outside the longitudinal excretory vessels. In *A. fuhrmanni* Skrjabin, 1915 (40) from an unknown host, the testes are disposed in two fields, genital pores are present in young segments and the uterine caecae do not lie outside the excretory vessels. Skrjabin (40) modified the generic diagnosis accordingly.

Taenia delafondi Railliet, 1892, in which the genital pores are almost always present, is included in the genus by Baer (1).   Thus, up to 1927, the species of *Aporina* formed a series, the genital pores being absent in *A. alba*, present in young segments in *A. fuhrmanni*, and usually present in *A. delafondi*.

In the same year, 1927, Meggitt (26) erected the genus *Killigrewia*, type species *frivola* and described another species *K. pamelae*. There are apparently no characters which distinguish *Killigrewia* from *Aporina* as envisaged by Skrjabin and Baer, and Meggitt does not discuss the affinities of his new genus.

Fuhrmann (11) made *Killigrewia* a synonym of *Aporina* and considered *K. pamelae* Meggitt, 1927 to be identical with *A. delafondi*.

Yamaguti (44) describes two new species from Formosa, *K. streptopoeliae* Yamaguti, 1935 and *K. onopopoeliae* Yamaguti, 1935.   Because of the presence of genital pores and the extent of the uterus and testes in his specimens, Yamaguti does not consider that they are congeneric with *Aporina alba*, thus presumably also not admitting *A. fuhrmanni* nor *A. delafondi* to the genus *Aporina*.

In 1934 Johri (15) reports *K. frivola* Meggitt, 1927 from India, upholding Meggitt's genus because of the arrangement of the testes in two fields, in contrast to *A. alba*, where they are in a continuous band.   No comment is made on the fact that there are two fields of testes in both *A. fuhrmanni* and *A. delafondi*.

It is proposed, therefore, to compare the present material with the described species of *Aporina* and *Killigrewia* from the Columbiformes and to consider them all as species of *Aporina*.

In 1941, *A. delafondi* was redescribed by Hussey (14) from North American material from *Columba livia* Gm. from Michigan.

As may be seen from Table III, this Egyptian material differs distinctly from *A. fuhrmanni* in the length of the cirrus pouch and from *A. onopopoeliae* in the number of testes.

In *A. frivola* the upper limit given for the number of testes is slightly higher than that for the remaining two species and the Egyptian material.   The difference in this character between *A. streptopoeliae* and *A. delafondi* is very slight, and when further material from *Streptopelia* spp. of host is available, the former species of cestode may well prove to be identical with the latter.

Amoebotaenia brevicollis Fuhrmann, 1907

  Host: *Hoplopterus spinosus* L. (Charadriiformes) 2989; Faiyum. 3410;
          Beheira, collected by Mr. W. H. Wells.

This worm was originally described by Fuhrmann (8) from *Charadrius nubicus* from Egypt and was redescribed by Joyeux and Baer (17).   The measurements given by the latter authors are shown in parentheses.

Several detached scoleces and two small worms, one lacking a head, were recovered.   The longer worm has a length of 2.5 mm. (2.3 mm.) and a maximum breadth of 0.5 mm. (0.73 mm.).

TABLE III

SPECIES OF *Aporina* FROM COLUMBIFORMES

Species	fuhrmanni	onopopoeliae	frivola	streptopoeliae	delafondi		
Author Described by:	Skrjabin, 1915 Skrjabin, 1915 (40)	Yamaguti, 1935 Yamaguti, 1935 (44)	Meggitt, 1927 Meggitt, 1927 (26)	Yamaguti, 1935 Yamaguti, 1935 (44)	(Railliet, 1892)* Baer, 1927 (1)	Hussey, 1941 (14)	Present material
Length	75 mm.	50 mm.	170 mm.	96 mm.	140 mm.	150 mm.	60 mm.
Breadth	6 mm.	4 mm.	5 mm.	5 mm.	5 mm.	5–6 mm.	4 mm.
Scolex	270 μ	150 μ	(280 μ Johri, 1934)	200 μ	220 μ	200 μ	182–189 μ
Suckers	103 μ	78 μ		75 μ	50 μ	110 μ	73–80 μ
Testes	Numerous	50	103–145	70–120	100 μ	71–101	93–114
Cirrus pouch	Unknown	230–250 ×50–75 μ	220–250 ×66–110 μ	200–250 ×80–100 μ	180 μ	350 μ	240–263 ×44–47 μ
Host		Streptopelia tranquebarica humilis (Temm.)	Unknown Columba livia intermedia Strick. Johri, 1934	Streptopelia chinensis formosa (Kuroda)	Columba livia Gm. Ectopistes migratoria (L.) Columba gymnophthalmus Temm. Streptopelia senegalensis senegalensis (L.) Europe, Asia, Africa, America	Columba livia Gm.	Columba livia Gm.
Locality	E. Bolivia	Formosa	Egypt	Formosa		Michigan	Egypt

NOTE: For abbreviations, see Table I.
*Syn. *Killigrewia pomedae* Meggitt, 1927, Fuhrm., 1932.

TABLE IV

SPECIES OF *Angularella*

Species	*taiwanensis*	*ripariae*	*beema*	
				Present material
Author	Yamaguti, 1940	Yamaguti, 1940	(Clerc, 1906)	—
Described by:	Yamaguti, 1940 (45)	Yamaguti, 1940 (45)	Clerc, 1906 (5)	—
Length	7-12 mm.	8-11 mm.	45 mm.	—
Breadth	1.1-1.75 mm.	1.4-1.75 mm.	1.2 mm.	—
Scolex	200-240 μ	300-350 μ	300 μ (250-300 μ Joyeux & Baer, 1936)	292-335 μ
Suckers	50-55 μ	105-135 μ	—	73-102 μ
Rostellum	120 μ	72-80 μ	(120 μ Joyeux & Baer, 1936)	80-87 ×175 μ
No. of hooks	39-40	40	56	54
Length of hook	42-45 μ	27 μ	(ca. 60 Joyeux & Baer, 1936) 22-29 μ	22-24 μ ×18.5-21 μ
Testes	70-93	32-45	20-25 (25-30 Joyeux & Baer, 1936)	35-45
Cirrus pouch	120-170 ×60-78 μ	75-90 ×33-63 μ	(100 ×40 μ Joyeux & Baer, 1936)	168 ×22 μ
Host	*Hirundo daurica striolata* Temm. & Schleg.	*Riparia paludicola chinensis* (Grey)	"swallow", Urals (*Hirundo rustica* L.) *Clivicola riparia* L.	*Hirundo rustica* L. *Oenanthe isabellina* (Cretz.)
Locality	Formosa	Formosa	Europe	Egypt

NOTE: For abbreviations, see Table I.

The scolex has a diameter of 321 μ (384 μ) and the suckers of 145 μ. The rostellum measures 102 μ by 233 μ. There are 14 to 16 (16–20) rostellar hooks arranged in a single crown (Fig. 4). The hooks measure 55 to 63 μ (59–65 μ) by 32 to 38 μ.

The number of segments is small, only 14 (10–24) in the present specimen, which is a complete individual (Fig. 5). The genital pores are irregularly alternate. The testes number 13 to 18 (13–15) and the cirrus pouch measures 109 μ (104–240 μ) by 29 μ.

Angularella beema (Clerc, 1906)

 Hosts: Hirundo rustica L. (Passeriformes) 3407; Beheira. Collected by
   Mr. W. H. Wells.

   Oenanthe isabellina (Cretz.) (Passeriformes) 1538; Giza.

Several fragments of strobila and several detached scoleces were present.

The scolex has a diameter of 292 to 335 μ, and has four round suckers measuring 73 to 102 μ. The rostellum has a diameter of 80 to 87 μ across its expanded tip, and has a length of 175 μ. There are 54 rostellar hooks arranged in zig-zag fashion (Fig. 6). The hooks measure 22 to 24 μ by 18.5 to 21 μ.

The genital pores are irregularly alternate; the genital ducts pass dorsal to the excretory vessels. The testes, 35 to 45 in number, are situated posteriorly and extend right across the segment. The cirrus pouch, which is attenuated and not very muscular, measures 168 μ by 22 μ.

To date, only three species have been described for this genus. Their dimensions are given in Table IV.

Angularella sp.

 Host: Apus pallidus pallidus (Shelley) (Apodiformes) 1597; Giza.

Anomotaenia aegyptica (Krabbe, 1869)

 Host: Cursorius cursor cursor (Lath.) (Charadriiformes) 1521; Faiyum.

There was one scolex armed with a single crown of 20 hooks (Fig. 7) each measuring 26 to 27 μ in length.

The shape and size of the hooks agree with the description by Joyeux and Baer (17), who give 24 hooks, 24 to 28 μ long.

Anomotaenia dehiscens (Krabbe, 1879)

 Host: Passer domesticus (L.) (Passeriformes) 1425-1; Giza. 1721; Qena.
   3412; Beheira, collected by Mr. W. H. Wells.

There were two heads and several fragments of strobila.

The measurements given by Fuhrmann (10) for specimens from Switzerland are shown in parentheses.

The length of the worm was estimated at 40 mm. (15 mm.); the maximum breadth is 1 mm. (0.5–0.7 mm.).

The scolex, mounted in gum chloral has a diameter of 182 μ (250 μ): that of the suckers is 55 μ (120 μ) and of the rostellum 44 μ (36 μ). There are 20 to 24 (20) hooks measuring 14.5 to 16 μ (14 μ) by 12 to 13 μ (Fig. 8).
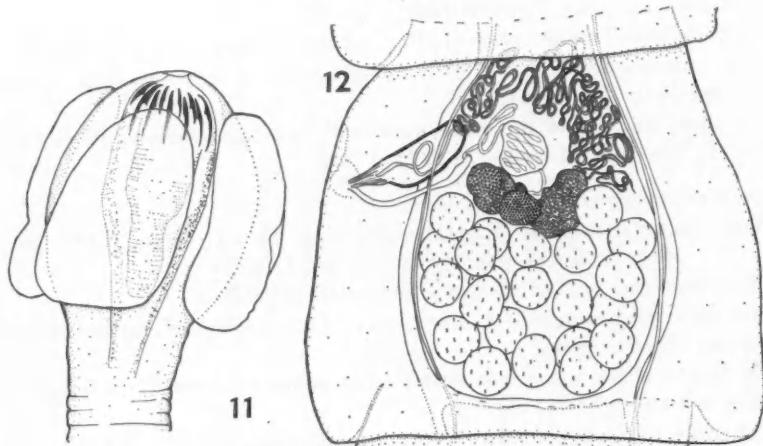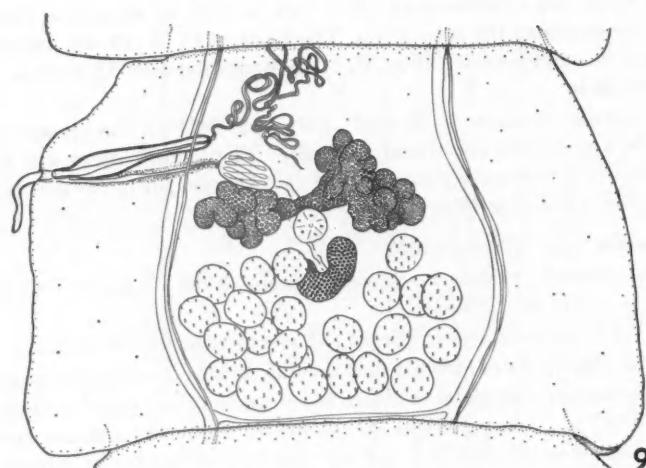
There are 20 to 25 (20–25) testes situated behind the ovary (Fig. 9) occupying the whole of the medulla between the excretory canals.   The cirrus pouch measures 182 to 204 μ (100 μ) by 36 μ.

The genital pores alternate irregularly; the genital ducts pass between the longitudinal excretory vessels.

The hooks also correspond in size and shape with those described and figured for *A. dehiscens* by Joyeux and Baer (17).   This worm was originally recorded by Krabbe from *Cinclus cinclus* L. from Turkestan.

Anomotaenia nymphea (Schrank, 1790)

Host: *Cursorius cursor cursor* (Lath.) (Charadriiformes) 1471, 1521; Faiyum.

The longest of the several specimens measures 35 mm. with a maximum breadth of 0.7 mm.

The scolex, mounted in Canada balsam, has a diameter of 310 to 366 μ with the rostellum withdrawn and of 225 μ with the latter evaginated (Fig. 11).   The rostellum has a diameter of 85 to 140 μ and a length of 282 to 380 μ. The suckers measure 277 to 343 μ by 87 to 146 μ.   There are 23 rostellar hooks arranged in a double crown (Fig. 10).   The large ones measure 87 to 94.5 μ by 36 to 40 μ, and the small ones 54.5 to 62 μ by 33 to 37 μ.

The genital pores alternate irregularly.   The testes number 22 to 25 and the cirrus pouch measures 124 to 146 μ by 50 to 73 μ in mature segments (Fig. 12).

The dimensions of the hooks agree most closely with those given for *A. nymphea* by Joyeux and Baer (17) viz., 20 to 24 hooks arranged in a double crown and with a length of 61 to 86 μ.

Dilepis sp.

Host: *Cursorius cursor cursor* (Lath.) (Charadriiformes) 1521; Faiyum.

Lateriporus merops Woodland, 1928

Host: *Merops apiaster* L. (Coraciiformes) 1607; Western Desert.

There was one specimen, the scolex of which was macerated and bore no hooks.   The rest of the anatomy, however, agrees with Woodland's original description (43) of *L. merops* from *Merops apiaster* L. from the Sudan.

This is the only species of the genus found in Coraciiformes.

Choanotaenia marchali (Mola, 1907)

Host: *Gallinula chloropus chloropus* (L.) (Ralliformes) 1892; Giza.

Three portions of strobila with heads and two unattached scoleces were present in the vial.   There were no gravid segments.

Fig.   7.   *Anomotaenia aegyptica*: rostellar hooks.
Fig.   8.   *A. dehiscens*: rostellar hooks.
Fig.   9.   *A. dehiscens*: dorsal view of mature segment.
Fig. 10.   *A. nymphea*: rostellar hooks.
Fig. 11.   *A. nymphea*: scolex.
Fig. 12.   *A. nymphea*: dorsal view of mature segment.

This species was redescribed by Joyeux and Baer (18) from the same host from the south of France. For purposes of comparison the measurements given by these authors are shown in parentheses.

The longest worm measures 25 mm. (30–50 mm.) and attains a maximum breadth of 0.5 mm. (1.5 mm.).

The scolex, mounted in Canada balsam, measures 310 to 451 μ (260–470 μ) and is provided with four suckers, 138 to 153 μ (135 μ) in diameter. The conical rostellum has a diameter of 87 to 104 μ (90 μ) and a length of 182 to 190 μ and is armed with 42 (42–46) hooks (Fig. 13) disposed in a double crown. The large hooks measure 32 to 34.8 μ (32 μ) by 21 to 22.7 μ and the small ones 25.9 to 27.5 μ (22–25 μ) by 17.8 μ to 19.4 μ.
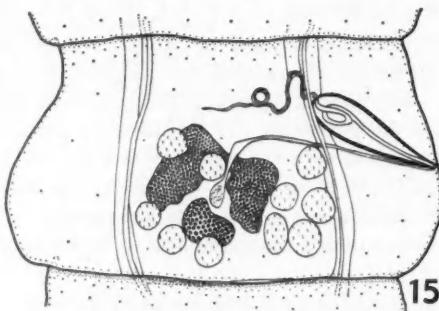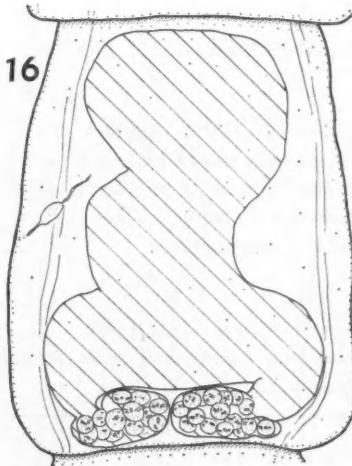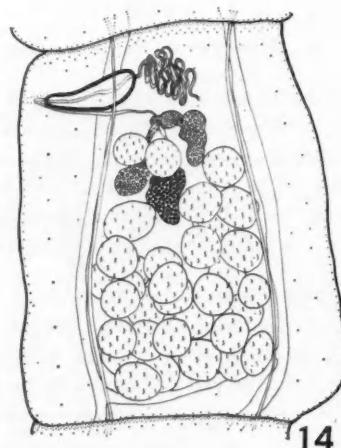
FIG. 13.   *Choanotaenia marchali*: rostellar hooks.
FIG. 14.   *C. marchali*: dorsal view of mature segment.
FIG. 15.   *Neyraia intricata*: dorsal view of mature segment.
FIG. 16.   *N. intricata*: dorsal view of ripe segment.

The genital pores are irregularly alternating. The testes are about 30 to 35 (45–50) in number, lying posteriorly in the segment, some behind the transverse commissure of the excretory system (Fig. 14). The cirrus pouch has a length of 109 μ (90–150 μ) and a breadth of 47 μ in mature segments. The tuft of setae at the base of the cirrus pouch is well developed, and is 80 to 87 μ long. There were no gravid segments.

There is a discrepancy in the number of testes in the two sets of material but this is not sufficient to constitute a specific difference when one considers the variation noted for the other species.

This is the only species of the genus recorded from Ralliformes.

Choanotaenia strigium Joyeux and Timon-David, 1934

Host: *Athene noctua* (Scop.) (Strigiformes) 1926; Giza.

The material, consisting of one portion of strobila, was macerated.

There are 30 to 35 testes; the cirrus pouch measures 120 to 127 μ by 25 to 29 μ. The details of the anatomy agree with the specimens originally described by Joyeux and Timon-David (22) from *Otus scops* (L.) collected near Marseille.

Only three species of *Choanotaenia* have been reported from owls: *C. strigium*, *C. speotytonis* Rausch, 1948 (36), *C. ululae* Mahon, 1954 (24).

Choanotaenia sp.

Hosts: *Burhinus senegalensis* (Sw.) (Charadriiformes) 1736; Qena.
       Hoplopterus spinosus L. (Charadriiformes) 2989; Faiyum.

Anonchotaenia globata (v. Linstow, 1879)

Hosts: *Anthus refulus* Vieill. (Passeriformes) 1473; W. Desert. 1522, 1524; Giza.
       Passer domesticus (L.) 2901; N. Cairo. "bird" 3138; Giza.

There were several entire specimens, the dimensions of which agree with those given by Joyeux and Baer (16) (given in parentheses) for *A. globata*, viz.; scolex diameter 690 to 708 μ (500–680 μ): diameter of suckers 282 to 296 μ (230–250 μ). The rostellum is lacking.

The genus is characterized by the little-marked external segmentation of the strobila, by the irregularly alternating genital pores, and by the development of a paruterine organ situated slightly porally. The testes number 5 (5) and are arranged in a straight line across the segment. The small cirrus pouch does not reach the poral excretory vessel and measures 54 by 25 μ (70–80 μ by 25 μ).

The genus *Anonchotaenia* is discussed by Rausch and Morgan (37) and by Voge and Davis (42). Most of the species occur in Passeriformes.

Anonchotaenia sp.

Hosts: *Monticola solitarius solitarius* (L.) (Passeriformes) 1816; Beheira.
       Oenanthe hispanica melanoleuca (Güldenstädt) (Passeriformes) 1563; Giza.

Neyraia intricata (Krabbe, 1882) Joyeux and Timon-David, 1934

   Syn. *Taenia intricata* Krabbe, 1882

   Biuterina lobata Fuhrmann, 1908

   Biuterina intricata (Krabbe, 1882) Moghe and Inamdar, 1934

   Biuterinoides upupai Ortlepp, 1940

   Host: Upupa epops L. (Upupiformes) 1814, 1862, 1906; Giza. 2078; Faiyum.

There were three incomplete specimens and several detached scoleces. One specimen, with scolex attached, has an estimated length of 150 mm. and a maximum breadth of 1 mm. The scolex is 592 μ in diameter, the suckers 212 μ, and the rostellum 141 μ in diameter. This material is macerated and the rostellar hooks have been lost. The second specimen consists only of gravid segments and is 48 mm. long. The scolex of the third specimen (preparation F) has a diameter of 564 μ, the suckers of 197 μ, and the rostellum measures 155 μ in width and 141 μ in length. The rostellar hooks, (it was not possible to count the total number) are arranged in four rows, the largest measure 34 to 36 μ by 16 to 21 μ, those of the next row 21 μ by 8 to 16 μ, the next 17 μ by 10 μ, and the fourth 9.7 μ.

The genital pores are irregularly alternating and the genital ducts pass between the excretory vessels. The testes number 8 to 10 (Fig. 15) and the cirrus pouch measures 73 μ by 44 μ. The uterus and paruterine organ are of the type characteristic for this species (Fig. 16). The embryos are large with a diameter of 47 to 55 μ. It was not possible to observe the egg shell. The embryonic hooks are large, 22 to 25.5 μ long.

A third detached head, mounted in Canada balsam, measures 451 μ in diameter, the suckers 141 μ, and the rostellum 155 μ by 127 μ.

These details of the anatomy agree with the redescription of *N. intricata* Krabbe, 1882, given by Joyeux and Timon-David in 1934 (21) from *Upupa epops* L. from the south of France (Table V).

The arrangement of the rostellar hooks is of particular interest and additional crush preparations (Fig. 17, A–E) of detached scoleces were made using gum chloral; drawings and measurements were made. Only those measurements made of the hook seen in profile are given. The dimensions along the axes *a* and *b* were used (Fig. 17A). The hooks are arranged in four alternating rows. Those of the first row have a triangular base surmounted by a small curved blade. Those of the second and third rows are more elongated and those of the fourth row are small and rather pear-shaped. There is considerable variation in shape and number, and a very wide variation in size (Table VI).

The genus *Neyraia* was erected in 1934 by Joyeux and Timon-David (21) to accommodate *Taenia intricata* Krabbe, 1882, and these authors give a redescription of the species based on material from *Upupa epops* L. from southern France. This genus is characterized by possessing four rows of hooks, which distinguishes it from *Biuterina* and the other paruterinines.

TABLE V

Neyraia intricata (KRABBE, 1882) JOYEUX AND TIMON-DAVID, 1934

Measurements given by:	Joyeux & Timon-David, 1934 (21)	Moghe & Inamdar, 1934 (31)	Orlepp, 1940 (34)	Present material
Length	105 mm.	50–55 mm.	110 mm.	150 mm.
Breadth	1.5 mm.	0.3 mm.	1.15 mm.	1 mm.
Scolex	400 μ	430 μ	720 μ	451–592 μ
Suckers	200 μ	—	240 μ	141–212 μ
Rostellum	170 μ	102 × 203 μ	168 × 120 μ	141–155 μ × 127–141 μ
No. of hooks	—	64	77	60–110
1st row	40–45 μ	52 μ	35–41 × 20–28 μ	29–47 × 16–32.5 μ
2nd row	30 μ	36 μ	24–30 × 11 μ	21–32.5 × 8–16.5 μ
3rd row	25 μ	30 μ	17–21 × 7–8 μ	16–29 × 6.5–13 μ
4th row	12 μ	19.6 μ	11–15 × 4.5–5 μ	8.1–17.8 × 4–9.7 μ
Testes	7–10	10	10	8–10
Cirrus pouch	90–100 × 32–37 μ	107 × 22 μ	120–132 × 42 μ	73 × 44 μ
Eggs	60–80 μ	27 × 22 μ	59–61 μ	—
Embryos	30 μ	14 μ	33–50 μ	47–55 μ
Embryonic hooks	22 μ	—	20–26 μ	22–25.5 μ
Host	Upupa epops L.	Upupa epops L.	Upupa epops L.	Upupa epops L.
Locality	South France	India	South Africa	Egypt

NOTE: For abbreviations, see Table 1.

TABLE VI

HOOK MEASUREMENTS OF Neyraia intricata (ALL MEASUREMENTS IN μ)

Preparation	Host No.	Mounting medium	1st row	2nd row	3rd row	4th row	Total no. of hooks
A Present material	2078	Canada balsam	29–33 × 18–23	23–28 × 9–11	16.5–19 × 7–8	9.7–13 × 5–8	110
F Present material	1864	Gum chloral	34–36 × 16–21	21 × 8–16	17 × 10	9.7	—
B Present material	1862	Gum chloral	35.6–37.3 × 22.7–25.9	25.9–30.8 × 10.6–13	17.8 × 11.3	8.1–10.6 × 6.5	69
C Present material	2078	Gum chloral	35–39 × 21–26.5	31–32 × 14.5–16.5	24 × 8	9.7–13 × 4–8	78
D Present material	2078	Gum chloral	35.6–39.7 × 26–27.5	29–32 × 13–16	22.7–29 × 9–13	14.5–17.8 × 9–9.7	60
Orlepp, 1940 (34)			35–41 × 20–28	24–30 × 11	17–21 × 7–8	11–15 × 4.5–5	77
Joyeux & Timon-David, 1934 (21)			40–45	30	25	12	—
E Present material	1862	Gum chloral	45–47 × 27.5–32.5	27.5–32.5 × 11.3–14.5	16–20 × 6.5–9.7	9.7–11.3 × 4–6.5	73
Moghe & Inamdar, 1934 (31)			52	36	30	19.6	64

From the same host from South Africa, Ortlepp in 1940 (34) described speci-
mens which he called *Biuterinoides upupai*.   Ortlepp felt it necessary to erect
a new genus distinct from *Biuterina* and it is evident from his discussion that he
was unaware of the paper by Joyeux and Timon-David; his material corres-
ponds with that of the latter authors (Table V).   Thus *Biuterinoides upupai*
Ortlepp, 1940 becomes a synonym of *Neyraia intricata* (Krabbe, 1882).
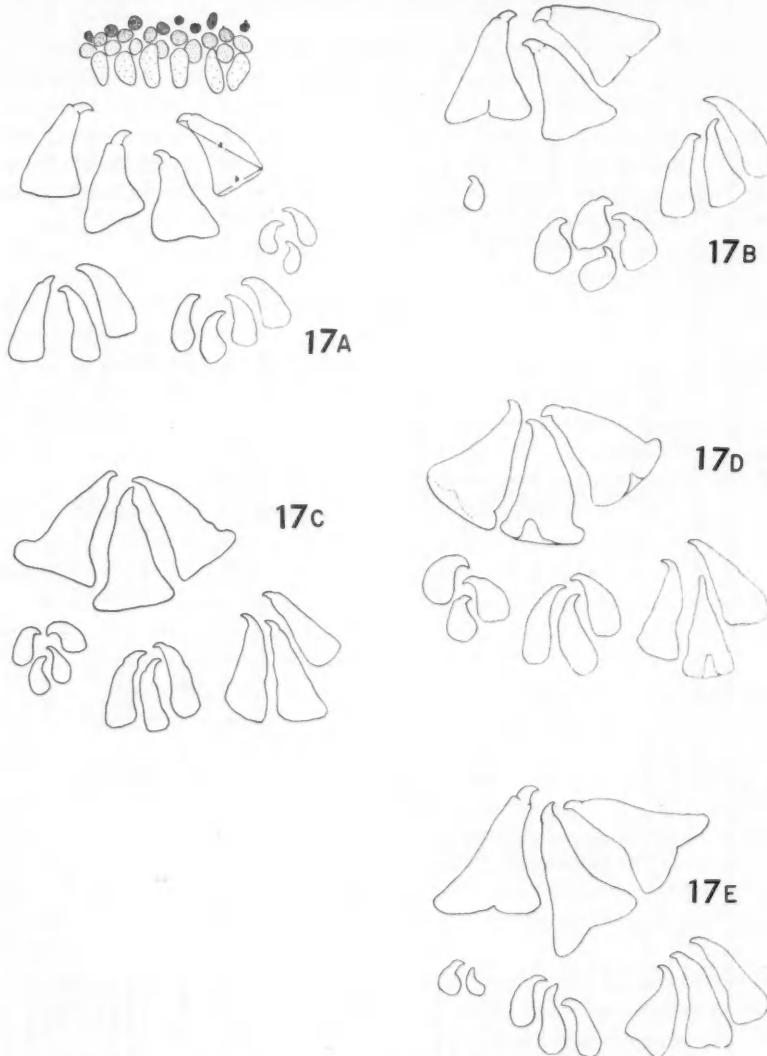
FIG. 17 A TO E.—*N. intricata*: rostellar hooks from five scoleces: all hooks drawn to
same scale.

In 1934, Moghe and Inamdar (31) described material from *Upupa epops* L.
which they considered to be identical with Krabbe's species *intricata* (Table V),
giving 52 μ, 36 μ, 30 μ, and 19.6 μ for the measurements of the hooks. As
may be seen for the present material from Egypt, the variation in hook size
and number is considerable. The limits for the Egyptian material are 29–47 μ
by 16–32.5 μ; 21–32.5 μ by 8–16.5 μ; 16–29 μ by 6.5–13 μ; 8.1–17.8 μ by
4–9.7 μ; total number of hooks 60–110 (Table VI). When measurements given
by Joyeux and Timon-David, 1934, Moghe and Inamdar, 1934 and Ortlepp,
1940 are included, the limits for hook length are 29–52 μ; 21–36 μ; 16–30 μ;
8.1–19.6 μ (Table VI). Within these limits, the hook dimensions form a series,
the terms of which may overlap (see Table VI). Thus, using hook measure-
ments as a criterion, there is no way of separating these forms into subgroups.
This wide range of hook measurements may imply one of two things. Either
the size and form of the hooks is not a distinguishing specific character, while
there would, however, be other anatomical differences of specific rank which
would allow the forms to be distinguished. This, however, cannot be deter-
mined in the present case because of the lack of material. Or, secondly, the
forms have not evolved into clearly differentiated species, and this state of
affairs is reflected in the lack of distinction in the hook characters. Inclining
to the second alternative, the Egyptian material is all ascribed to the species
Neyraia intricata, even though the variation in hook size is much greater
than that normally encountered within a single species.
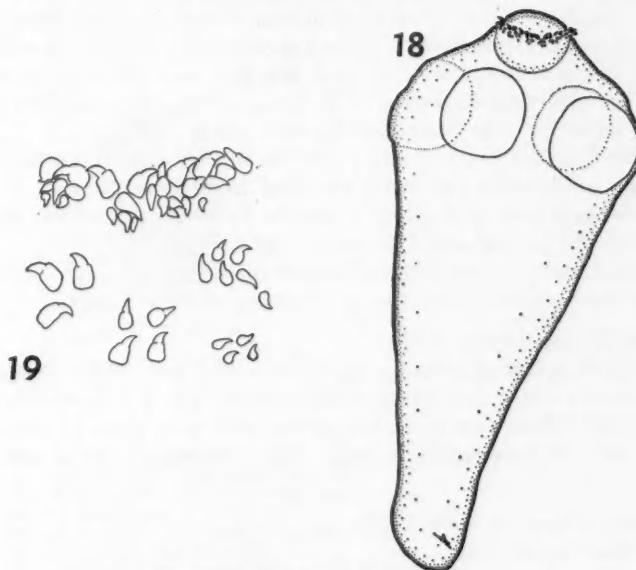
FIG. 18. *N parva* n. sp.: entire specimen.
FIG. 19. *N. parva* n. sp.: rostellar hooks.

Neyraia parva n. sp.

Host: Upupa epops L. Upupiformes 1125, 1906; Egypt.

There were two young specimens, one still bearing two embryonic hooks on its posterior end (Fig. 18). The other is only 2 mm. long, the widest part being the scolex. The neck is 1.1 mm. long and only the beginning of segmentation has occurred.

The scolex has a diameter of 338 to 387 μ. The round, unarmed suckers have a diameter of 127 by 99 μ and the small, armed rostellum a diameter of 92 to 99 μ and a length of 70 μ. There is a total of 86 hooks, arranged in four alternating rows. The largest hooks do not show the characteristic triangular shape, but have a rather blocklike base (Fig. 19). They measure 11.3 by 6 μ. Those of the second row have a shape similar to those of the first, and measure 9.7 by 4.9 μ. Those of the third and fourth rows are rather pear-shaped, and measure 6.7 by 4.1 μ and 4.8 by 3.2 μ respectively.

The shape, number, and size of the hooks show marked differences from *Neyraia intricata* (Krabbe, 1882) as described above, and although the anatomy of this worm must for the moment remain unknown, it seems justifiable to create a new species on the basis of the scolex characters.

Thus, to date, only two species of *Neyraia* are known, both described from the bird, *Upupa epops* L., but from widely separated localities, viz., Egypt, south of France, South Africa, and India.

Diorchis longicirrosa Meggitt, 1927

Host: Anas crecca crecca L. (Anseriformes) 2860; Qalyubiya.

A single specimen of a small, delicate worm, 15 mm. in length and 0.5 mm. in maximum breadth, possessing a scolex but no rostellar hooks, was examined.

The scolex has a diameter of 160 μ. The oval suckers measure 94 μ by 109 μ. The rostellum is 65 μ wide. The cirrus pouch extends past the median line of the segment. The two testes are both aporal (Fig. 20).

The genus *Diorchis* is discussed by Schultz in 1940 (38) and a key is also given. This author lists 14 species described from Anseriformes. *D. longicirrosa* is distinguished from all other species by its short strobila, less than 50 mm. long, and by the aporal location of both testes.

The original material described by Meggitt (27) is from *Anas crecca crecca* L. (Anseriformes) and *Fulica atra atra* L. (Ralliformes) from Egypt.

Haploparaxis filum (Goeze, 1782)

Host: Capella gallinago gallinago (L.) (Charadriiformes) 2839; Giza.

There was one scolex, bearing 10 rostellar hooks (Fig. 21), measuring 17.8 μ by 10 to 12 μ. These dimensions correspond with those given by Joyeux and Baer (17) viz., 10 hooks, 18.5 μ long. For a discussion of this species see Davies (7).

Hymenolepis bilharzi (Krabbe, 1869)

Host: "bird" 3138; Giza.

The head bears 10 rostellar hooks of characteristic shape (Fig. 22), measuring 16 μ by 14.5 μ. The length of hook given by Joyeux and Baer (17) is 14 to 16 μ.
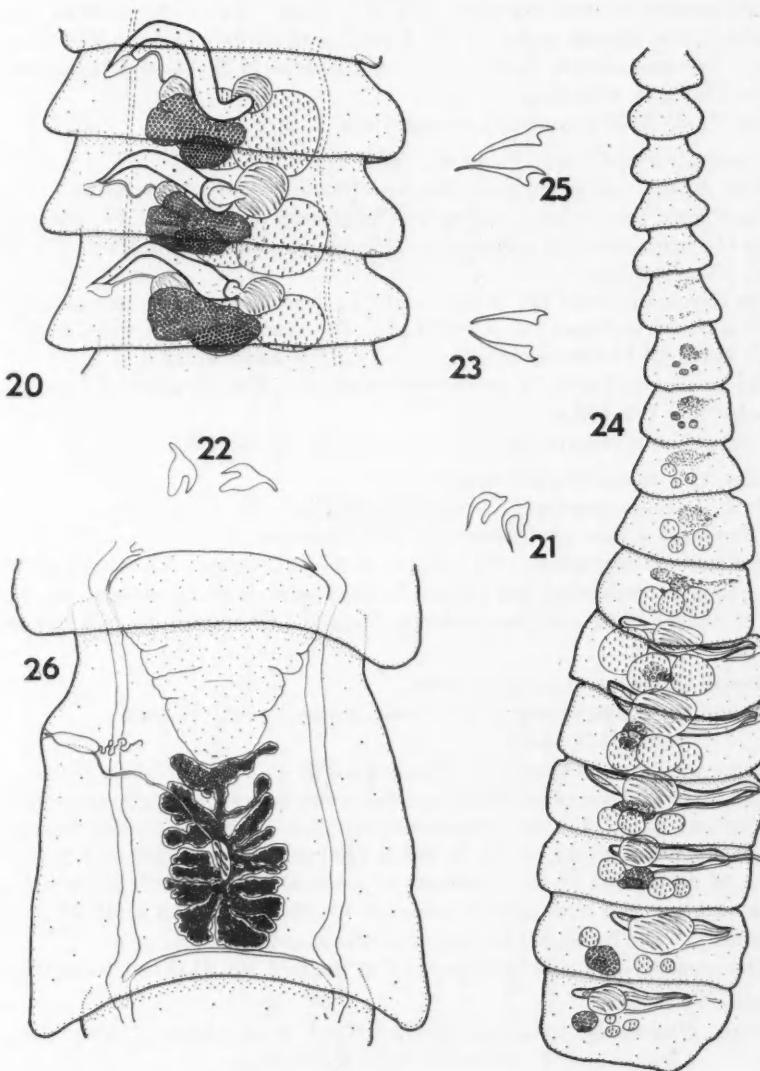
FIG. 20. *Diorchis longicirrosa*: ventral view of mature segments.
FIG. 21. *Haploparaxis filum*: rostellar hooks.
FIG. 22. *Hymenolepis bilharzi*: rostellar hooks.
FIG. 23. *H. fringillarum*: rostellar hooks.
FIG. 24. *H. pauciannulata*: dorsal view of strobila.
FIG. 25. *H. stylosa*: rostellar hooks.
FIG. 26. *Cladotaenia* sp.: dorsal view of gravid segment.

This species occurred together with *H. stylosa*. As all the scoleces were detached, it is difficult to decide which portion of strobila belongs with which head. Previous records are by Joyeux and Baer in 1928 (16) and Joyeux and Timon-David in 1934 (22).

The "bird" 3138 is probably a passeriform.

Hymenolepis fringillarum (Rudolphi, 1809)

   Host: Lanius senator niloticus (Bonap.) (Passeriformes) 1608; Giza.

There were two scoleces and several fragments of strobila. For the purposes of comparison the measurements given by Joyeux and Baer (17) are given in parentheses.

The scolex, diameter 127 to 145 μ (300 μ) bears four suckers measuring 54 to 58 μ by 76 to 79 μ (110 μ by 90 μ). The rostellum, 55 μ wide, bears a single crown of 10 (10) hooks (Fig. 23), 26 μ (26–28 μ) by 22 μ.

The two aporal testes lie one behind the other. The cirrus pouch measures 54 μ by 40 μ (95–100 μ).

This species is reported by Joyeux and Gaud in 1945 (20).

Hymenolepis pauciannulata Meggitt, 1927

   Host: Anas querquedula L. (Anseriformes) 2979; Egypt.

There were several specimens, none with scoleces.

The worm is very small, with a length of 0.7 to 1.1 mm. The cirrus pouch, 150 μ long, extends across the segment to the aporal excretory vessels (Fig. 24).

The material originally described by Meggitt (27) is from *Spatula clypeata* (L.) from Egypt.

Hymenolepis stylosa (Rudolphi, 1809)

   Hosts: Muscicapa striata striata (Passeriformes) 1201; N. Sinai.
              "bird" 3138; Giza.

There were several specimens with heads, but with no gravid segments.

The shape and size of the hooks and the scolex agree with the description by Joyeux and Baer (17): the dimensions they give are shown in parentheses.

The diameter of the scolex is 167 μ (200 μ), of the suckers 72 to 80 μ (85 μ by 60 μ), and of the rostellum 43 μ (30 μ). The latter has a length of 83 μ, and is armed with a single crown of 10 (10) hooks, 27.5 μ (28–35 μ) by 23 μ long. The hooks have a characteristic shape (Fig. 25).

The presence of *H. stylosa* indicates that the bird No. 3138 is a passeriform.

Hymenolepis spp.

   Hosts: Phoenicurus ochrurus ochrurus (Gm.) (Passeriformes) 1580; Giza.
              Corvus corone L. (Passeriformes) 1673; Qena.
              Monticola saxatalis (L.) (Passeriformes) 1630; Giza.
              "bird" (Passeriformes) 3126; Faiyum.

Cladotaenia sp.

   Hosts: Falco biarmicus tanypterus Schleg. (Accipitriformes) 1800; Giza.
              Falco tinnunculus tinnunculus L. 1593; Giza.

There were four scoleces but all had lost their hooks. The longest specimen measures 120 mm. and has a maximum breadth of 1.4 mm.

TABLE VII

SPECIES OF *Cladotaenia*

Species	*Cladotaenia banghami*	*Cladotaenia foeta*	*Cladotaenia circi*	*Cladotaenia* sp.
Author	Crozier, 1946	Meggitt, 1933	Yamaguti, 1935	Present material
Measurements taken from:	Crozier (6)	Meggitt, (30)	Yamaguti, (44)	
Length	150–250 mm.		133 mm.	120 mm.
Breadth	1.4 mm.		1.83 mm.	1.4 mm.
Scolex	286.5 μ	190 μ	200 μ	240–365 μ
Suckers	148 ×12 μ		80 μ	109–146 ×131–204 μ
Rostellum	137 μ	130 μ	42 ×72 μ	44–80 ×153–248 μ
No. of hooks	36		48	Lost
Large hook	36–39.6 μ		24 μ	–
Small hook	28.8–29.4 μ		18 μ	–
Testes	105–111	85–97	90–110	140
Cirrus pouch	73–159 ×38–134 μ	120–160 ×50–70 μ	120–150 ×50 μ	182–219 ×66 μ
No. uterine branches	11–19	17–21	7–10	ca. 7
Lateral fields of testes	Joined	Joined	Joined	Joined
Extent of uterus	Reaches genital pore	Past genital pore	Past genital pore	Reaches genital pore
Host	*Haliaetus l. leucocephalus* (L.)	*Circus assimilis* Jardine & Selby	*Circus a. aeruginosus* (L.)	*Falco biarmicus tanypterus* Schleg. *F. t. tinnunculus* L.
Locality	Ohio, N. America	Calcutta zoo	Formosa	Egypt

NOTE: For abbreviations, see Table 1.

The scolex has a diameter of 240 to 365 μ. The suckers measure 109 to 146 μ by 131 to 204 μ and the rostellum is 44 to 80 μ wide and 153 to 248 μ long.

The testes number about 140 and the majority are disposed in two lateral fields on either side of the female organs. The lateral fields are joined by a row of testes posterior to the ovary. The cirrus pouch measures 182 to 219 μ by 66 μ.

In the gravid segment (Fig. 26), the uterus extends forward to the level of the genital pore, giving off about seven pairs of lateral branches from the main stem. Anterior to the uterus, the medullary parenchyma shows a particular modification, rather resembling the tissue of a paruterine organ. This character is remarked upon by Fuhrmann and Baer in 1943 (12) in their redescription of *Cladotaenia melierax* Woodland, 1929 from *Melieras gaber* (Daud.) from the Sudan.

The members of this genus (Crozier (6)) appear to be restricted to accipitriform birds, save *C. mirsoevi* Skrjabin and Popov, 1924, inadequately described, reported from a mammal and also C. *secunda* Meggitt, 1928 (28) from an unknown host from Egypt.

From those species described from Africa, viz. *C. cylindracea* (Bloch, 1782); *C. armigera* (Volz, 1900); *C. melierax* (Woodland, 1929); *C. freani* Ortlepp, 1938 (33) and *C. vulturi* Ortlepp, 1938 (33), the present material differs in the number and arrangement of the testes and the forward extent of the uterus.

The disposition of the testes and the forward extent of the uterus in the gravid segment may be used to differentiate species-groups. In the three species *C. banghami*, *C. circi*, and *C. feuta* (Table VII) the lateral testicular fields are joined by a row of testes posterior to the ovary and the non-alveolar uterus extends forward to the level of the genital pore, as is the case in the present specimens. The measurements for these species are given in Table VII, together with those of the Egyptian material and as may be seen, the number of testes and of uterine branches are not very good specific criteria, at least when considered alone. It is unfortunate that the present material is without the rostellar hooks. As the other dimensions do not fit clearly into any of these three species and as there are also differences in host distribution, it is preferable merely to ascribe this material to the genus *Cladotaenia* without making a specific determination.

Undeterminable Fragments

Fragments of worms from the following hosts could not be identified.

Phoenicurus phoenicurus phoenicurus (L.) (Passeriformes) 1202; N. Sinai.

Phoenicurus sp. 1252; S. Sinai.

Apus pallidus pallidus (Shelley) (Apodiformes) 1606; Giza.

Merops apiaster L. (Coraciiformes) 1653; Red Sea Coast.

Monticola saxatalis (L.) (Passeriformes) 1610, 1613; Giza.

Motacilla flava L. (Passeriformes) 3031; W. Desert.

Passer domesticus L. (Passeriformes) 1919; W. Desert. 1425; Giza. 1458-1-2; Faiyum.

Host List

AVES

ANSERIFORMES
  Anas crecca crecca L.
  Anas querquedula L.

            Diorchis longicirrosa Meggitt, 1927
            Hymenolepis pauciannulata
              Meggitt, 1927
            Tetrabothrius sp.

ACCIPITRIFORMES
  Falco biarmicus tanypterus Schleg.
  Falco tinnunculus tinnunculus L.

            Cladotaenia sp.
            Cladotaenia sp.

GALLIFORMES
  Coturnix sp.

            Raillietina (*Fuhrmannetta*)
              malakartis n.sp.

RALLIFORMES
  Gallinula chloropus chloropus (L.)

            Choanotaenia marchali (Mola,
              1907)

CHARADRIIFORMES
  Burhinus senegalensis (Swainson)
  Cursorius cursor cursor (Lath.)

            Choanotaenia sp.
            Anomotaenia aegyptica
              (Krabbe, 1869)
            Anomotaenia nymphea
              (Schrank, 1790)
            Dilepis sp.

  Hoplopterus spinosus L.

            Amoebotaenia brevicollis
              Fuhrmann, 1907
            Choanotaenia sp.

  Capella gallinago gallinago (L.)

            Haploparaxis filum (Goeze, 1782)

COLUMBIFORMES
  Columba livia Gm.
  Streptopelia senegalensis senegalensis (L.)

            Aporina delafondi (Railliet, 1892)
            Cotugnia polycantha Fuhrmann,
              1909
            Raillietina (*Raillietina*) *fuhrmanni*
              idiogenoides (Baer, 1933)

STRIGIFORMES
  Athene noctua (Scop.)

            Choanotaenia strigium
              Joyeux and Timon-David, 1934

APODIFORMES
  Apus pallidus pallidus (Shelley)

            Angularella sp.

CORACIIFORMES
  Merops apiaster L.

            Lateriporus merops Woodland,
              1928

UPUPIFORMES
  Upupa epops L.

            Neyraia intricata (Krabbe, 1882)
            Neyraia parva n. sp.

PASSERIFORMES
  Anthus refulus Vieill.

            Anonchotaenia globata (v. Linstow,
              1879)

  Corvus corone L.
  Hirundo rustica L.
  Lanius senator niloticus (Bonap.)

            Hymenolepis sp.
            Angularella beema (Clerc, 1906)
            Hymenolepis fringillarum
              (Rudolphi, 1809)

  Monticola saxatilis (L.)
  Monticola solitarius solitarius (L.)
  Muscicapa striata striata

            Hymenolepis sp.
            Anonchotaenia sp.
            Hymenolepis stylosa
              (Rudolphi, 1809)

Oenanthe hispanica melanoleuca (Güldenstädt)
*Oenanthe isabellina* (Cretzschmar)
Passer domesticus (L.)

Anonchotaenia sp.
Angularella beema (Clerc, 1906)
Anomotaenia dehiscens
    (Krabbe, 1879)
Anonchotaenia globata
    (v. Linstow, 1879)
Raillietina (Raillietina) galeritae
    (Skrjabin, 1915)

Phoenicurus ochrurus ochrurus (Gm.)
"passeriform" (3138)

Hymenolepis sp.
Hymenolepis bilharzi
    (Krabbe, 1869)
Hymenolepis stylosa
    (Rudolphi, 1809)
Anonchotaenia globata
    (v. Linstow, 1879)

"bird" (3126)

Hymenolepis sp.

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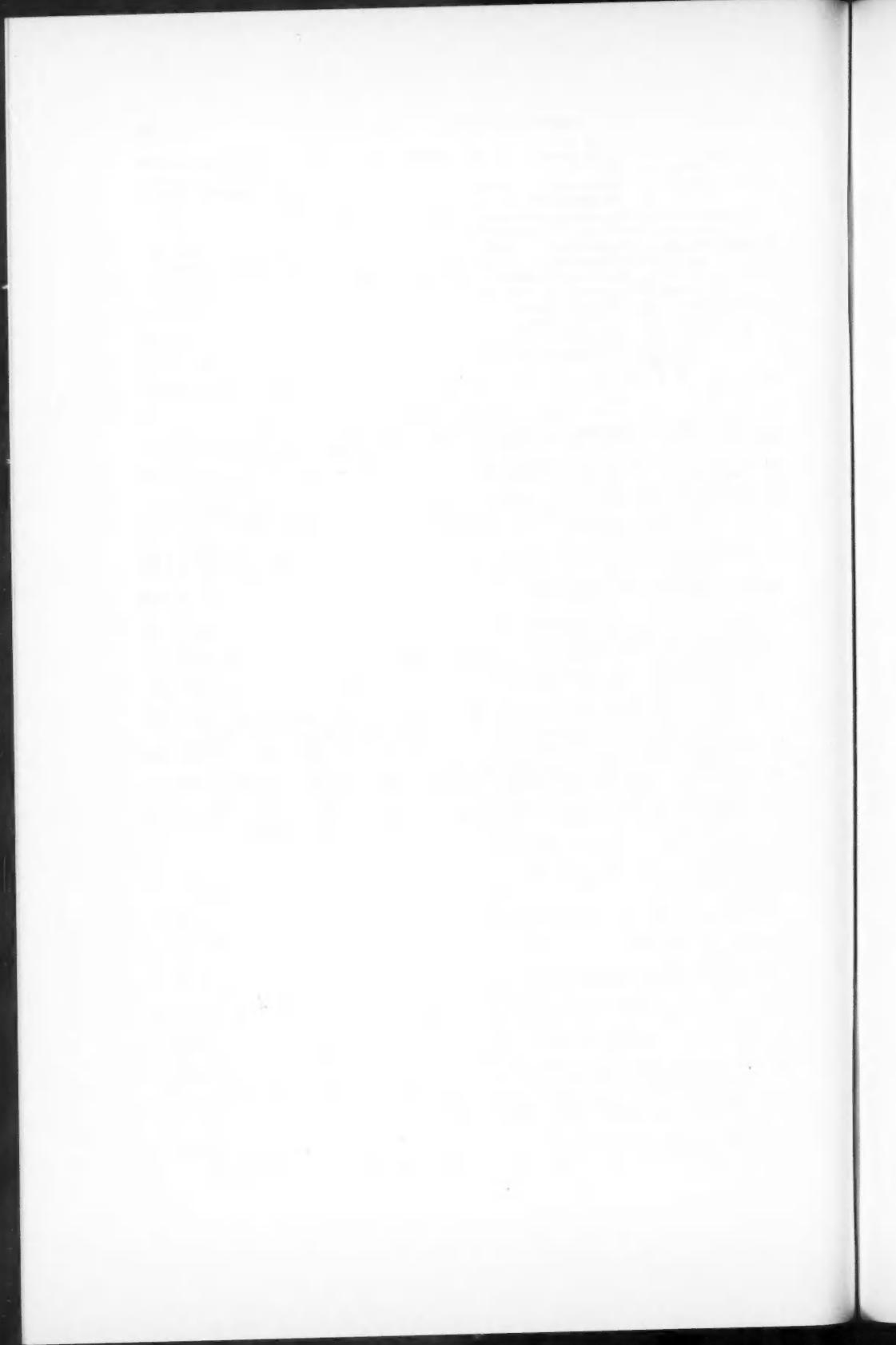
THE BIOLOGY OF THE TROPICAL SEA URCHIN TRIPNEUSTES ESCULENTUS LESKE IN BARBADOS, BRITISH WEST INDIES[1]

JOHN B. LEWIS[2]

Abstract

Tripneustes esculentus is a large white-spined sea urchin, common at Barbados and in coastal waters throughout much of the tropical Atlantic, which is exploited locally as food. Its biology is given here in terms of growth, reproduction, development, reactions, methods of feeding, and variability. Growth as a whole is relatively rapid during fall and winter months, but slows down during spring and summer, which is the period of gonad maturation. Ripening individuals tend to aggregate and spawning occurs throughout the three summer months. The larvae are described and also the metamorphosis of late planktonic stages. Adult animals move away from sources of light stimulus. They feed almost exclusively upon algae.

Introduction

Tripneustes esculentus Leske is a littoral sea urchin living on rocky bottom from low water to a depth of three or four fathoms. It is widely distributed in the West Indies, occurring from Bermuda and the Carolina coast to the east coast of Mexico and Central America, through the West Indies to Brazil and at Ascension and the west coast of Africa. It is the largest of the regular echinoids in the area, a test diameter of 14 to 15 cm. being not uncommon. It occurs in considerable numbers around the coast of Barbados and is most abundant in areas of loose broken rock and on rock flats which support a heavy growth of algae. It is missing from areas of sand bottom and seldom occurs on an actively growing coral reef.

Tripneustes is of some economic importance to the island of Barbados. A well-established fishery for the animals has existed for many years (9). The gonads of both sexes are considered a delicacy and large numbers of urchins are fished each year and sold as food. So great is the demand for them that the Barbados Government has instituted a closed season for the fisheries during the summer months from May to September.

It is of interest that the other regular tropical echinoid, *Lytechinus variegatus* Leske, which has a similar widespread distribution and is of similar habits, does not occur at Barbados. A doubtful record of the species in Barbados has been given by Clark (1) but no specimens have been seen by the author.

Methods

From November of 1954 through September of 1957, representative monthly samples of 50 to 100 urchins were collected from a shallow water area. All urchins which occurred along the line of a transect from the shore to the edge

of the coral reef were collected. Since the larger urchins tend to move out from the shore during at least part of the year, this method of selection insured that all size groups were sampled. Test diameter and test height of each individual were measured with calipers.

In September of 1956, a collection of newly settled urchins was placed in a live trap in the sea and retained during the ensuing year. Freshly collected algae (*Zonaria*, *Padina*, and *Dictyota* spp.) were placed in the trap three times a week. Once a month the urchins were removed from the trap for measurement and then replaced.

During the 3-year period, monthly samples of 30 mature individuals were taken from a number of areas. Test diameter and height, gonad volumes, and gonad condition were recorded from these samples. In addition a small number of individuals of smaller size were examined each month for gonad condition. Gonad condition was estimated from examination of a smear of fresh gonad on a microscope slide. Females were recorded as ripe if the smear contained 90% mature ova. Males were recorded as ripe if the smear contained predominantly active spermatozoa. This method of estimating degree of ripeness has been used by Moore (8) for *Echinus esculentus*. Ripe gonads of both sexes will run freely when the test is broken and this activity was helpful also in estimating ripeness.

In the summer of 1957 a series of plankton hauls was made in coastal waters to obtain information on the larval ecology and early development of the species. In all, a total of 47 hauls were made, beginning May 6th and concluding on September 15th. Most hauls were made during the morning hours between 0800 and 1000 hours and each haul was of 7 to 10 minutes' duration. Plankton nets were made of nylon, 48 mesh per inch or 96 mesh per inch, 18 in. in diameter. The fine mesh net was used for below-surface hauls (10 to 15 meters) and the medium mesh net for surface hauls.

Growth

The size distribution curves derived from the monthly samples are shown in Fig. 1. Fig. 1 (top) is the family of curves for the period November 1954 to September 1955; Fig. 1 (middle), the curves for October 1955 to September 1956; and Fig. 1 (bottom), the curves for the period October 1956 to September 1957.

The distribution curves for the months of September of each year are all bimodal and it is evident that two populations are represented. The lower peaks on curves in the 1 to 3 cm. range represent the newly settled juvenile urchins. These juveniles first appear under rocks and in sheltered crevices in August and then in abundance during the early part of September.

The second peak of the September curves, in the 5 to 8 cm. range, represents the population which settled during the previous year and is the spawning population of the current year. The peaks of the curves of this year-old group are smoothed out or disappear during the following months. This is

due to the fact that the season during which the urchins may be fished opens on the first of September of each year. By October or November the year-old group has been fished out and the population in most localities consists only of the juveniles which had settled during the summer.
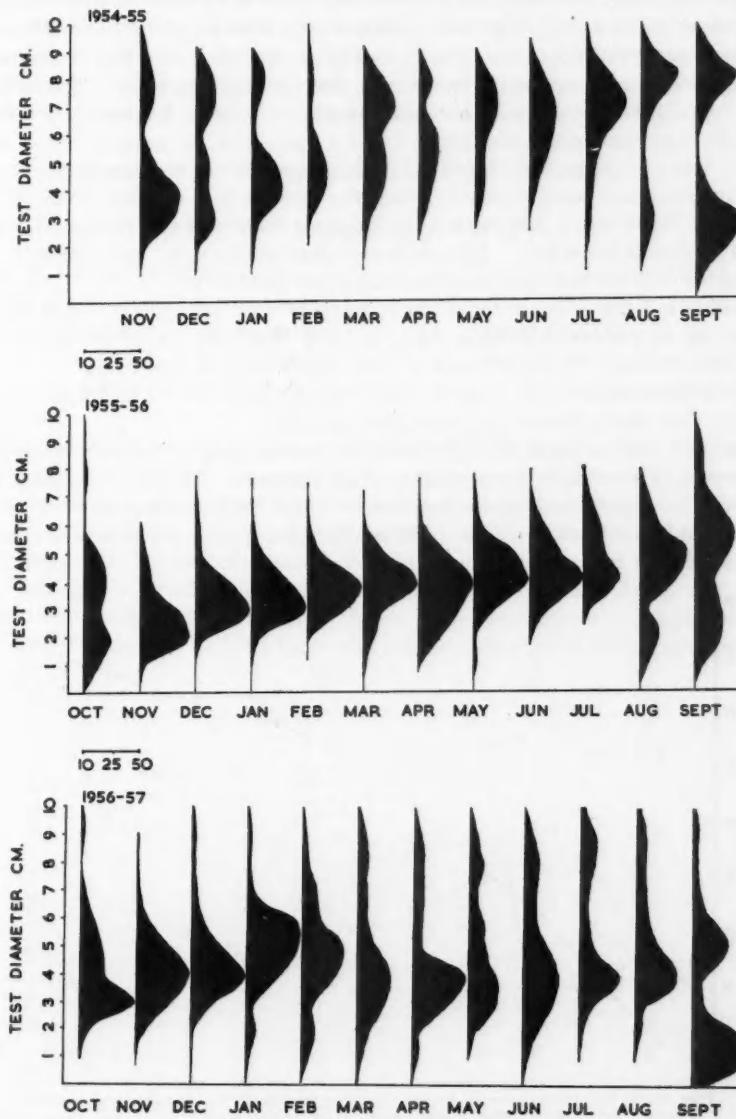

Fig. 1. Size distribution of *Tripneustes esculentus*.

In 1954–55 (Fig. 1, top) the peak of the distribution curve in November was between 3 and 4 cm.   By May of 1955 urchins had reached a test diameter of 7 cm.   There was no evidence of further growth during June and July but in August the population had reached a size of 8 cm.

In 1955–56 (Fig. 1, middle) the peaks of the curves in October and November lie between 2 and 3 cm.   There was a gradual increase in size up until March at which time the population had attained a size of 4 cm. test diameter. Again there was no apparent increase in size through the following months until August when the size increased sharply to 5 cm.   In September the population reached a size of 6 cm.

In October of the period 1956–57 (Fig. 1, bottom) there was a peak at 3 cm. Growth continued until February when the urchins had reached a size of 5 to 6 cm.   There was a sudden drop in the peak value to 4 cm. during March, which is difficult to explain.   Size peaks remained at this value until September of 1957 when there was a sudden increase in size to 6 cm.

Considering the 3 years together, it appears that growth is rapid from September of each year through February and March of the following years. The curves of Fig. 1 indicate that urchins which were of 1 to 3 cm. test diameter in September reach a size of 4 to 6 cm. by April of the following year. Growth then slows down from April through July.

The period from April through July and part of August corresponds with the period of growth and maturation of the gonads.   At the completion of spawning in August and September there is again an increase in growth rate. By September of each year the urchins which are a year old have reached a size range of 6 to 8 cm. test diameter.
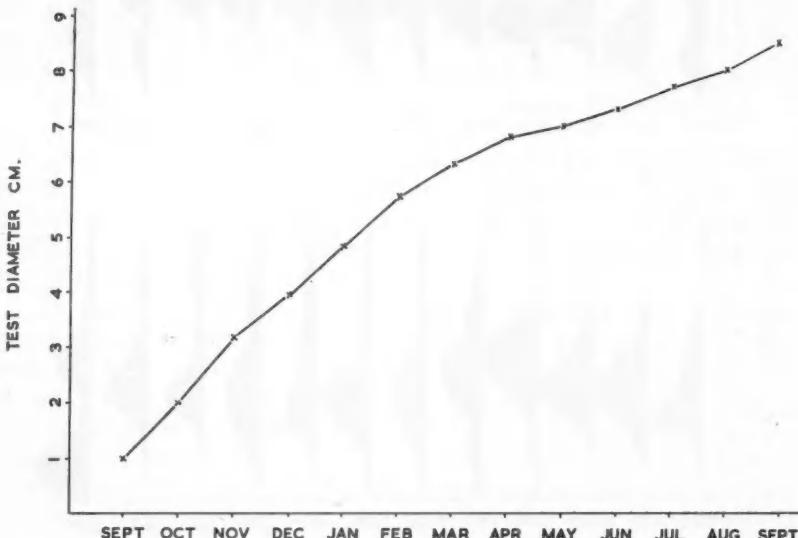

FIG. 2.   Mean monthly test diameters of *Tripneustes esculentus* kept in a live trap.

The growth rate of urchins which were kept in a live trap in the sea is shown in Fig. 2. The sample of juveniles placed in the trap in September of 1957 had a mean test diameter of 1.1 cm. By the onset of the breeding season at the beginning of May they had reached a size of 6.8 cm. During the breeding season the growth was slowed and mean test diameter of 7.8 cm. was reached by the end of August. During the month of September the growth rate was increased slightly and a mean size of 8.2 cm. was reached.

The slightly higher growth rates of urchins in the live trap are to be expected. Not only did these urchins receive an abundance of food but there was no disappearance of the larger specimens due to fishing as in the case of the monthly samples on which the curves of Fig. 1 are based.

Breeding

At the height of the breeding season the gonads of both sexes of *Tripneustes* are large and occupy almost the full volume of the test. In females the gonads are bright orange in color; in males they are a lighter yellow. These color differences persist to a lesser degree throughout the period of gonad maturation. At the conclusion of spawning the gonads of both sexes become darker in color.

The monthly variation in gonad volumes for 1955, 1956, and 1957 is shown in Fig. 3. The volumes are expressed as a ratio, gonad volume/test diameter. In 1955 the gonads of both sexes began to increase in volume in February and reached full size in July. Spawning was completed by the end of August and the gonad volumes decreased sharply during this month. In 1956 the gonads began to mature in March, reached a peak volume in April, and continued full volume through July. As in 1955 spawning was completed by the end of August. In 1957 there was a sharp increase in gonad volumes in
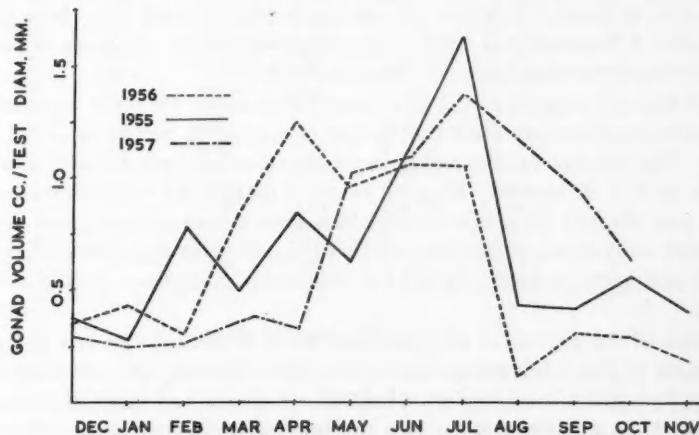

FIG. 3. Seasonal change in gonad volumes of *Tripneustes esculentus*.

May.   They continued to increase in volume slowly through the month of July and decreased in September.   Thus in 1957 the breeding season was prolonged through the end of August and into September.

Although growth and maturation of gonads continues in some cases through the summer most urchins are ripe and actively spawning by June.   This is shown by the data of Fig. 4.   In this figure the percentages of ripe, non-ripe, and spent individuals, for the spawning season of 1956, is shown.   In January 30% of the sample consisted of individuals which had ripe eggs.   No spent individuals were taken until March but during March and April 5–10% of the sample consisted of spent individuals.   The percentage of unripe urchins decreased in March and April to 50 and 12% respectively and by June the population consisted only of ripe and a few spent urchins.   At the time of the August sample (August 12th) 80% of the sample consisted of spent individuals, indicating that the bulk of the spawning took place throughout July and in early August.
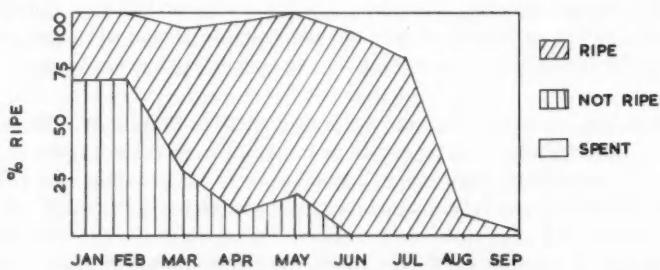
FIG. 4.   Seasonal change in gonad condition of *Tripneustes esculentus* during 1956.

Further information on the breeding period was obtained from the incidence of larvae in the series of plankton hauls made twice a week from May until the middle of September of 1957.   The occurrence of the numbers of plutei and of metamorphosing larvae is shown in Table I.

Plutei began to appear in the plankton at the end of May and were taken in considerable numbers until the middle of August, a period of about ten weeks.   The greatest numbers of larvae were taken between the end of June and the middle of August.   Keeping in mind that gonad volumes increased in this year through July, it is evident that some spawning took place before the gonads reached maximum size and that the peak spawning period occurred in July and early August.   Spawning was nearly completed by the end of August.

Records of the growth of the gonads of small individuals are not included in the data of Fig. 3 but casual observation indicated that they were sexually mature during the same period.   Individuals which had reached a size of only 2 to 3 cm. test diameter in May or June had full gonads containing ripe eggs and sperm.

TABLE I

NUMBERS OF *Tripneustes* LARVAE TAKEN IN PLANKTON HAULS DURING THE SUMMER OF 1957

Date		Haul No.	Time	Depth	Net	Length of haul	No. larvae per haul	
							Plutei	Metamor.
May	6	A	1000	10 m.	Fine	7 min.	0	0
	15	B	1000	10 m.	Fine	7 min.	0	0
	24	1	1000	Surf.	Fine	10 min.	15	15
	27	2	1000	10 m.	Fine	10 min.	60	4
June	3	3	0900	10 m.	Fine	10 min.	0	0
	3	4	0900	Surf.	Fine	10 min.	32	0
	7	5	0830	10 m.	Fine	7 min.	8	0
	14	6	1430	10 m.	Fine	7 min.	8	4
	14	7	1430	Surf.	Fine	7 min.	4	4
	20	8	0900	Surf.	Fine	7 min.	0	8
	20	9	0900	10 m.	Fine	7 min.	8	4
	25	10	0900	5 m.	Fine	10 min.	4	28
	25	11	0900	Surf.	Coarse	10 min.	2	50
	25	12	0900	Surf.	Fine	10 min.	1	68
	26	13	0900	Surf.	Fine	10 min.	5	30
	26	14	0900	Surf.	Coarse	10 min.	0	22
	26	15	0900	10 m.	Fine	10 min.	32	8
July	8	16	0930	15 m.	Fine	10 min.	10	15
	8	17	0930	Surf.	Coarse	10 min.	0	5
	10	18	0930	Surf.	Coarse	10 min.	1	17
	10	19	0930	15 m.	Fine	10 min.	15	16
	15	20	0930	Surf.	Coarse	10 min.	1	14
	15	21	0930	15 m.	Fine	10 min.	55	10
	19	22	0930	Surf.	Coarse	10 min.	0	187
	19	23	0930	15 m.	Fine	10 min.	20	95
	23	24	0900	Surf.	Fine	10 min.	0	15
	23	25	0900	Surf.	Coarse	10 min.	0	14
	30	26	0830	Surf.	Coarse	10 min.	0	30
	30	27	0830	10 m.	Fine	10 min.	84	4
Aug.	6	28	0830	5 m.	Fine	10 min.	5	65
	6	29	0830	Surf.	Coarse	10 min.	0	2
	10	30	0830	Surf.	Coarse	10 min.	0	6
	10	31	0830	10 m.	Fine	10 min.	92	0
	12	32	1430	15 m.	Fine	10 min.	244	8
	15	33	0830	10 m.	Fine	10 min.	0	0
	15	34	0830	Surf.	Coarse	10 min.	0	0
	19	35	0830	10 m.	Fine	10 min.	4	0
	19	36	0830	Surf.	Coarse	10 min.	0	0
	23	37	0900	Surf.	Coarse	10 min.	0	1
	23	38	0900	10 m.	Fine	10 min.	16	0
	29	39	1430	Surf.	Coarse	10 min.	2	2
Sept.	2	40	0900	Surf.	Coarse	10 min.	2	2
	2	41	0900	10 m.	Fine	10 min.	1	1
	7	42	0830	Surf.	Coarse	10 min.	3	2
	7	43	0830	10 m.	Fine	10 min.	0	1
	15	44	0900	10 m.	Fine	10 min.	1	1
	15	45	0900	Surf.	Coarse	10 min.	1	1

Spawning Behavior

The fact that the presence of sexual products in the water will stimulate ripe echinoids to spawn has been noticed by a number of authors (3, 10). It was found that *Tripneustes* brought into the laboratory will spawn if ripe within one or two hours. However, treatment with sexual products brought

on spawning much more quickly. Direct application of eggs or sperm to the area of the test around the genital apertures did not cause spawning immediately but a solution of active sperm mixed with sea water around the urchins caused both males and females to spawn within a few minutes. Similar treatment with a solution of eggs was less effective.

During the months of March and April there was a noticeable decrease in the numbers of urchins living on the upper surfaces of rocks and on grassy flats. Individuals became crowded together in groups of several to a dozen under rocks and ledges. Few specimens could be seen out in the open from April through July. In August, mature individuals were again found moving about in considerable numbers on the surface of the bottom and the large aggregations were dispersed.

The tendency of ripening urchins to aggregate in groups is of obvious importance for the successful fertilization of eggs. The stimulation to spawning of large groups at one time would be provided by the initial spawning of one or two urchins.

Fertilization and Early Development

The eggs of *Tripneustes* are light orange in color, 0.08 mm. in diameter, and sink in water. During fertilization, active sperm became attached in a thick layer to the eggs. The first cleavage was completed in an hour and fifteen minutes; the four-celled stage was reached in two hours. Blastulae were formed within twelve hours and swimming gastrulae within twenty hours. Early stage plutei were formed within two days. Larvae at this stage (Fig. 5A) have a short simple gut and are 0.29 mm. in width. They are transparent with their edges dotted with fine carmine pigment granules. They are positively phototropic. A pair of short postoral arms tipped with carmine forms during the third day.

Although numerous attempts were made to rear larvae in fine mesh cages of the type used by Thorson (12) in dishes of running sea water and in jars to which cultures of ciliates had been added, none were successful. Under such conditions larvae lived only for 7–10 days. However, various developmental stages were taken from the plankton and from these the period of larval life was estimated.

Figure 5 shows the various stages in the development of the plutei from a 2-day-old to a mature larva. A 7-day larva, Fig. 5B, is 0.44 mm. long, bears a pair of long postoral arms, and on the oral lobe the developing buds of the anterolateral arms. The larva is transparent and is colored with the characteristic scattered carmine pigment granules.

Figure 5C shows an intermediate stage in which the preoral arms have reached a length equal to that of the anterolateral and a short pair of posterolateral arms are present. A pair of short ciliated epaulettes on the shoulder of the posterior region have begun to form. This stage is 0.65 mm. in length.

A fully developed pluteus larva is shown in Fig. 5D. It is 0.8 mm. in length, transparent, and heavily pigmented. The echinus rudiment at the posterior end near the base of the posterolateral lobes is a light green in

color and shows up in striking contrast to the body of the pluteus in living material.   The two posterior lobes each bear a well-developed ciliated epaulette.   There are four anterior epaulettes.

From the data of Table I it appears that the length of development from fertilized egg to mature pluteus is about one month.   The young larvae first appear in numbers in plankton hauls at the end of May.   Metamorphosing larvae appear just a month later, the last week in June.

Metamorphosing larvae were common in the plankton from the end of June until the end of August.   They were more common in surface hauls than at a depth of 10–15 meters.   From the abundance of metamorphosing larvae in the plankton it is apparent that development normally continues in the plankton beyond the mature pluteus stage.   Figures 6A and 6B show dorsal and ventral views of a larva which is in the process of metamorphosis.
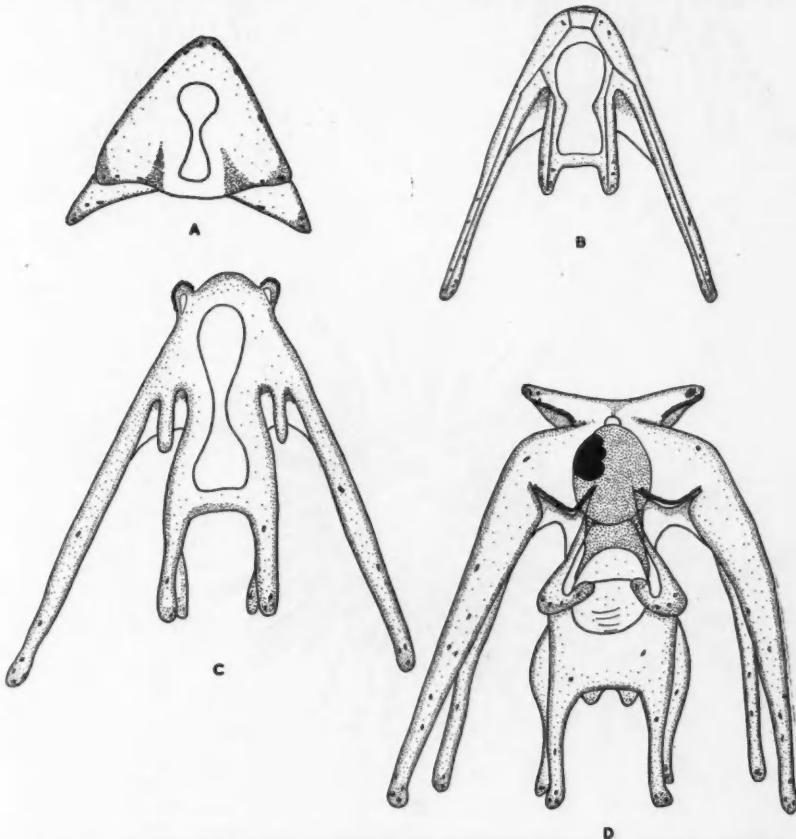

FIG. 5.   Larvae of *Tripneustes esculentus*.   A. Two-day-old pluteus.   B. Seven-day-old pluteus.   C. Intermediate stage.   D. Mature pluteus.

A

B

C
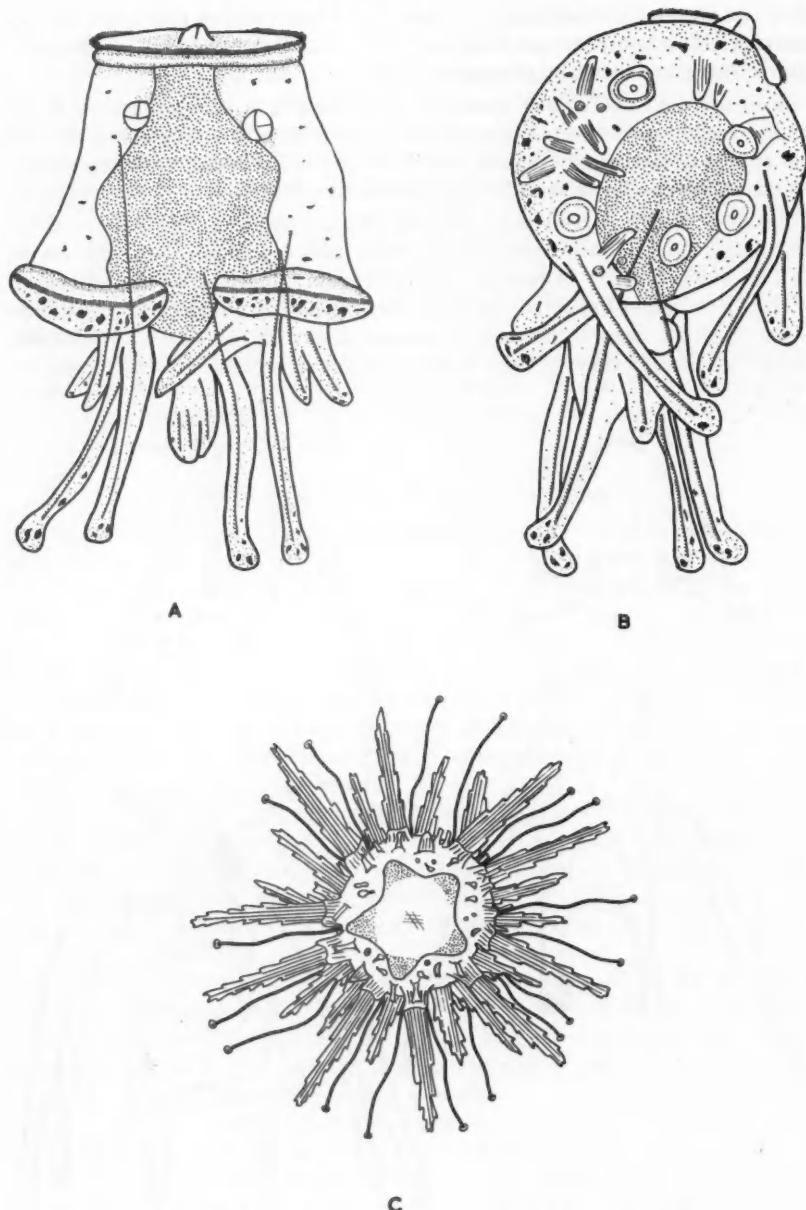
FIG. 6. Larvae and juvenile of *Tripneustes esculentus*. A and B. Metamorphosing larvae. C. Juvenile 3 weeks old.

The larva is 1.0 mm. in length and still retains the remains of the pluteal arms and skeleton. The central portion of the body is a light-green color and carmine pigment granules have become concentrated around the edge of the body. The five primary tube feet are formed and are functional and there are three primary pedicellariae. A number of short stout spines are present.

This larva still retains the power of swimming and indeed in observed living material this power appears to be increased over that of a mature pluteus.
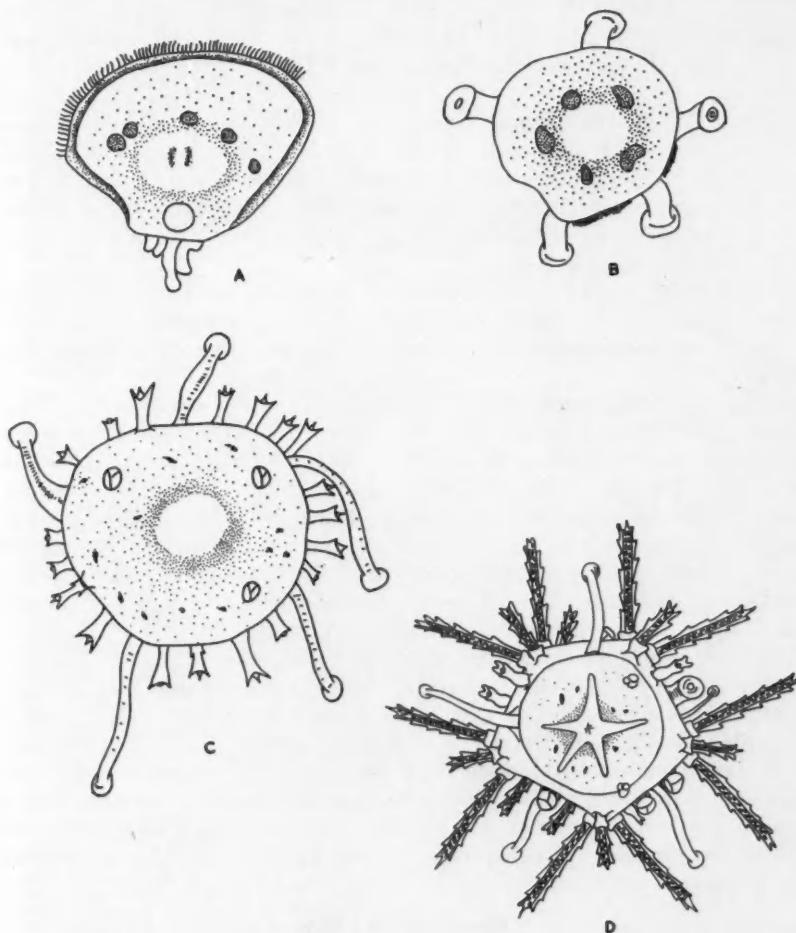
FIG. 7. Larvae and juveniles of *Tripneustes esculentus*. A and B. Degenerate metamorphosing larvae. C. Juvenile settling stage 1 day old. D. Juvenile settling stage 9 days old.

The ciliated bands are still retained but have come to lie in a position different from that which they occupied in the pluteus. The posterior epaulettes at the base of the posterolateral processes have come to lie in a single fused band which encircles the posterior end of the body. The four anterior epaulettes of the pluteus have fused in the metamorphosing larva and lie in two bands around the anterior end of the body. These larvae swim posterior end foremost.

Although larvae were obtained in various stages of metamorphosis, those of Figs. 6A and 6B were by far the commonest. This fact would suggest that this stage is the final one in the development in the plankton before the larva finds suitable settling grounds. It is admirably suited as a 'searching' stage having both the power of locomotion and tube feet for attachment to a substrate.

A series of metamorphosing larvae were kept in the laboratory to gain information on their settling behavior. A control group was placed in 3-in. diameter petri dishes, 25 per dish, covered with 98 mesh per inch bolting silk, under a regulated drip of sea water. Test groups were placed under similar conditions in dishes containing, respectively, fresh ground algae, sand, and fragments of rock.

There appeared to be no difference in preference for different types of substrate among the separate groups. Including the control group, 90% of the larvae completed metamorphosis and became juvenile urchins within twelve hours.

A series of metamorphosing larvae was also kept in dishes of sea water which were allowed to stand without agitation and with a change of water only every two or three days. Of these, 50% completed metamorphosis in from one to two days while the other half remained as swimming larvae for periods up to two weeks before they died. During this time there was absorption of the body tissues and larvae of the types shown in Figs. 7A and 7B were formed. Both these types were taken in the plankton and it seems likely that such larvae are degenerate forms which have had a prolonged larval life without reaching suitable settling grounds.

Juvenile stages which develop after settling are shown in Figs. 6 and 7. Figure 7C is a juvenile (0.6 mm. test diameter) 1 day old from settling time. Figure 7D is a juvenile (0.7 mm. test diameter) 9 days old and Fig. 6C, a juvenile (1.6 mm. test diameter) 3 weeks old.

A small number of later individuals were obtained from metamorphosing larvae liberated in aquaria. Over a period of eleven weeks these reached a size of 3–6 mm. test diameter. Figure 8 is a photograph of a juvenile of 4 mm. test diameter. At this stage all were clearly recognizable as juveniles of *Tripneustes esculentus*.

Reaction to Light

It has been shown by a number of workers that a variety of echinoids are sensitive to light. In general they are negatively phototropic and tend to move into shaded areas or under rocks and into the sand. This reaction

PLATE I
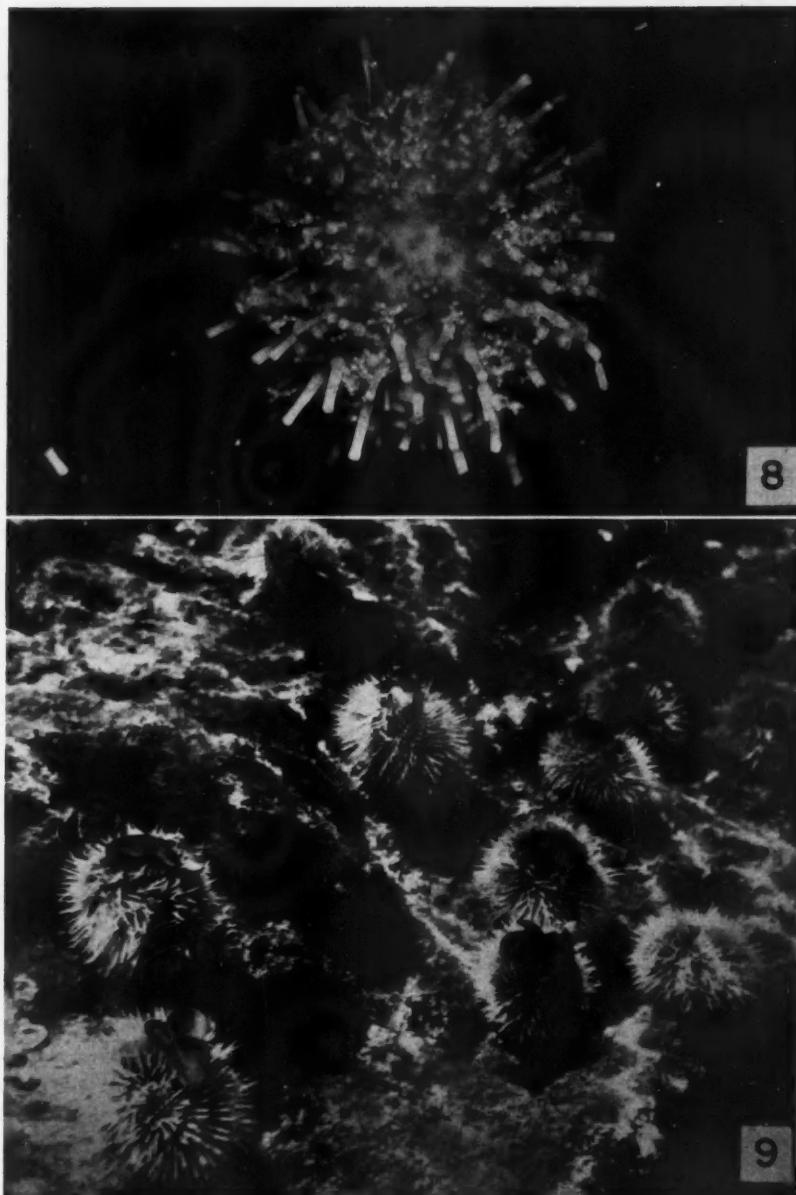
FIG. 8. Photograph of a juvenile *Tripneustes*, 4 mm. test diameter.
FIG. 9. Underwater photograph of a group of *Tripneustes* showing urchins covered with algae and other debris.
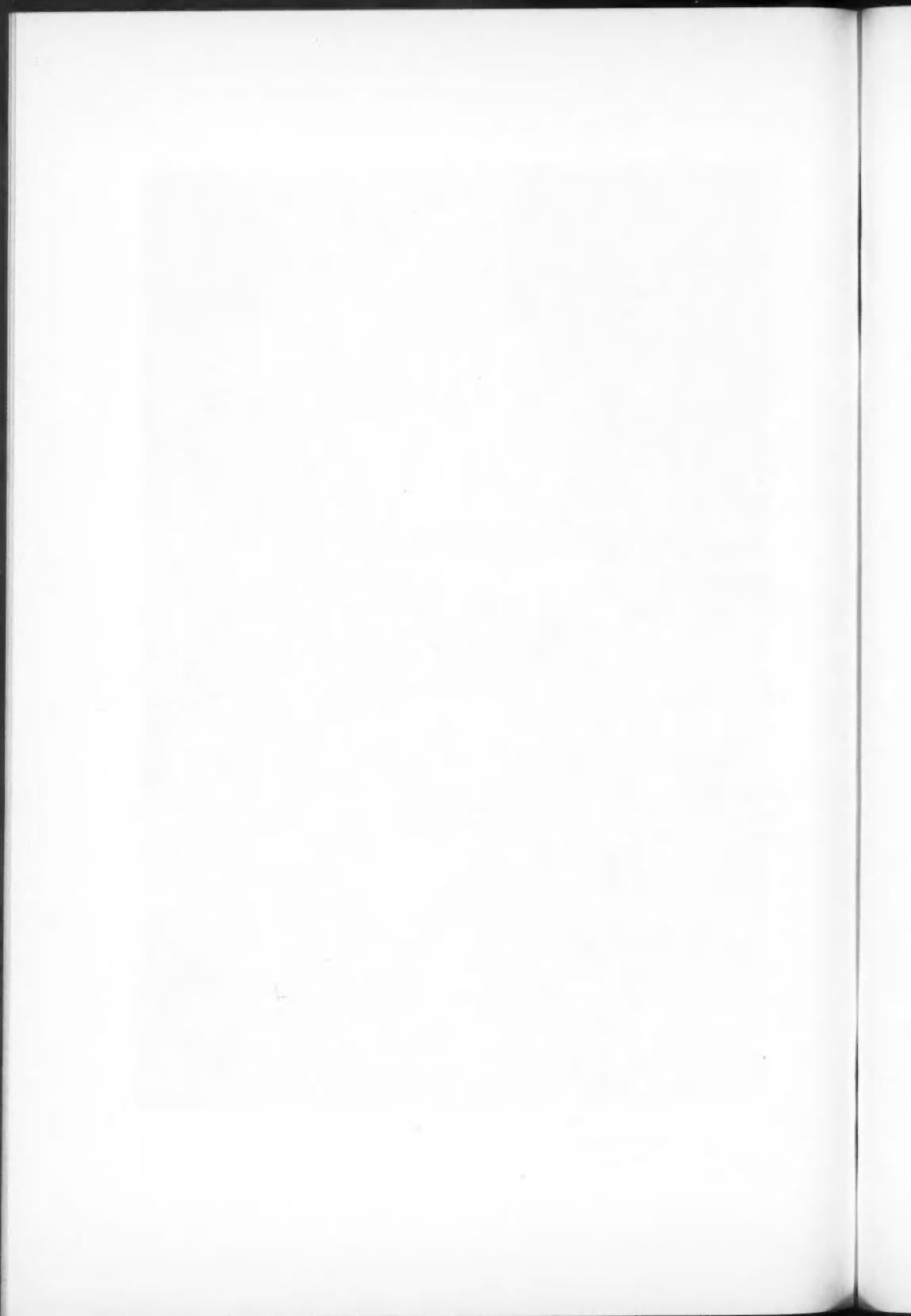
has been shown for *Psammechinus lividus* by Dubois (2), for *Arbacia punctulata* by Mangold (5), and for the tropical echinoid *Diadema setosum* by Millott (6, 7).

In its natural habitat *Tripneustes* dwells beneath loose rocks and other objects.   It is also found in areas of eel grass and low algae where there is no cover.   In such situations it is invariably covered with·fragments of shell, stone, or other debris.   The fragments are held by the tube feet and the spines, and the urchin is often completely covered by the debris (see Fig. 9).

Urchins kept in shallow water tables and in aquaria retreated to shaded corners and most remained passive during the greater part of the day.   Thus both in its natural habitat and in the laboratory, the avoidance of light is a characteristic form of behavior.

In the laboratory *Tripneustes* shows responses to both direct illumination and changes in light intensity.   The responses to change in light intensity apply to both increase and decrease in illumination.

When a shadow was cast upon an urchin or·diffuse light shone upon it there was an immediate erection of the spines followed by a circular waving motion.   This was accompanied by waving of the pedicellariae and an opening and shutting of their jaws.   An extension of the tube feet followed, principally on the side opposite the source of light change.   The tube feet were also waved about as in a searching motion and movement of the whole animal away from the light or cast shadow took place.

The response to direct illumination from a spotlight took ˙two forms. Violent waving of the spines, pedicellariae, and tube feet took place over the whole surface of the test surrounding the illuminated area.   In specimens which had no covering of debris there was a sharp movement of the animal away from the light.   With a strong light, fatigue occurred quickly and specimens often remained stationary after a period of five or ten minutes with only slow movements of the appendages.

In specimens which were partially covered with pieces of algae or stone, there was a co-ordinated movement of the spines and tube feet after the initial waving.   Individuals were placed in a shallow water table with a piece of algae covering half the test.   A spot light from a microscope lamp was shone upon the opposite side of the test from the algae.   The piece of algae was then carried by means of spine movement and pulled by the tube feet across the test to cover the illuminated area.   Slow movement of the animals away from the light often accompanied this reaction but a large proportion of individuals merely remained stationary and transported the algae to the lighted area.   Switching of the fragment back and forth several times on movement of the light spot occurred before the animals became fatigued and ceased to react.

The covering of *Tripneustes* with fragments of debris is evidently a light-avoiding reaction.   Specimens which were collected from under rocks or in deeply shaded areas were seldom covered.

Food and Feeding

Tripneustes feeds almost exclusively upon algae. Gut examination of 50 individuals from several localities revealed a variety of species of algae but nothing of an identifiable animals' nature.   However, specimens which were kept in aquaria in a starved condition were observed to feed upon dead limpets and polychaetes. Of a number of species of algae offered to urchins in the laboratory there was a preference for the broad leafy forms such as *Padina* and *Dictyota* spp. Algae which contained a high proportion of calcareous material such as *Halimeda* were refused and there was also rejection of an *Enteromorpha*.

Under certain conditions *Tripneustes* appears to be a detritus feeder, for numerous specimens examined had the gut packed only with fine particles of calcareous sand.

Tripneustes is capable of detecting food at some distance. Specimens which had remained on a shallow water table for a day or two without food were observed to detect food placed in the table up to 12 inches away.   A handful of algae was placed in the table in such a position as to cast no shadow upon the urchins. After several minutes there was a waving of the spines and an extension and waving of the tube feet in the direction of the food. The urchins then began to move out of shaded corners into the light and towards the food. In some there was a direct line and in others a more random route and a certain amount of trial-and-error searching.

Color Variation

There was no noticeable external color difference between the two sexes. Among younger specimens of the first-year group there was more color variation than among second-year individuals. In first-year specimens the spines may be tipped with rose, have an olive-green base with white or rose tips, have a rose base with lighter brown at the tips, or have pure white spines. The pedicellariae in these cases are always dark brown and the tube feet are light brown, olive, or reddish. The test varies from dark brown to olive green.

In older specimens the spines are usually white, occasionally tipped with rose.   The test and pedicellariae are dark brown to near black and the tube feet dark brown, occasionally white.

A light phase occurs in 1–2% of the population.   In this type the spines are white, tube feet cream or light olive, the pedicellariae are light, and the ambulacral membrane a very light green to cream.   This light phase occurred in all size groups and there was no correlation with sex.

Less than half a dozen specimens of a very dark phase were taken from a cave on a single occasion. The spines and pedicellariae of this form were dark red and the tube feet and test a lighter red.

External Parasites and Commensals

Two animals, a decapod macruran and a small gasteropod, were commonly found on the test and spines of *Tripneustes*. The macruran, *Gnathophylloides minerii* Schmitt, is a small commensal which is found clinging to the spines of the urchins and is often very abundant. A description of the animal has been published by Schmitt (11) and further notes on coloration, breeding, and feeding habits were made by Lewis (4).

A small gasteropod of the family Melanellidae also occurs upon the test. Urchins which were infested with the snail had areas of the test bared of spines.

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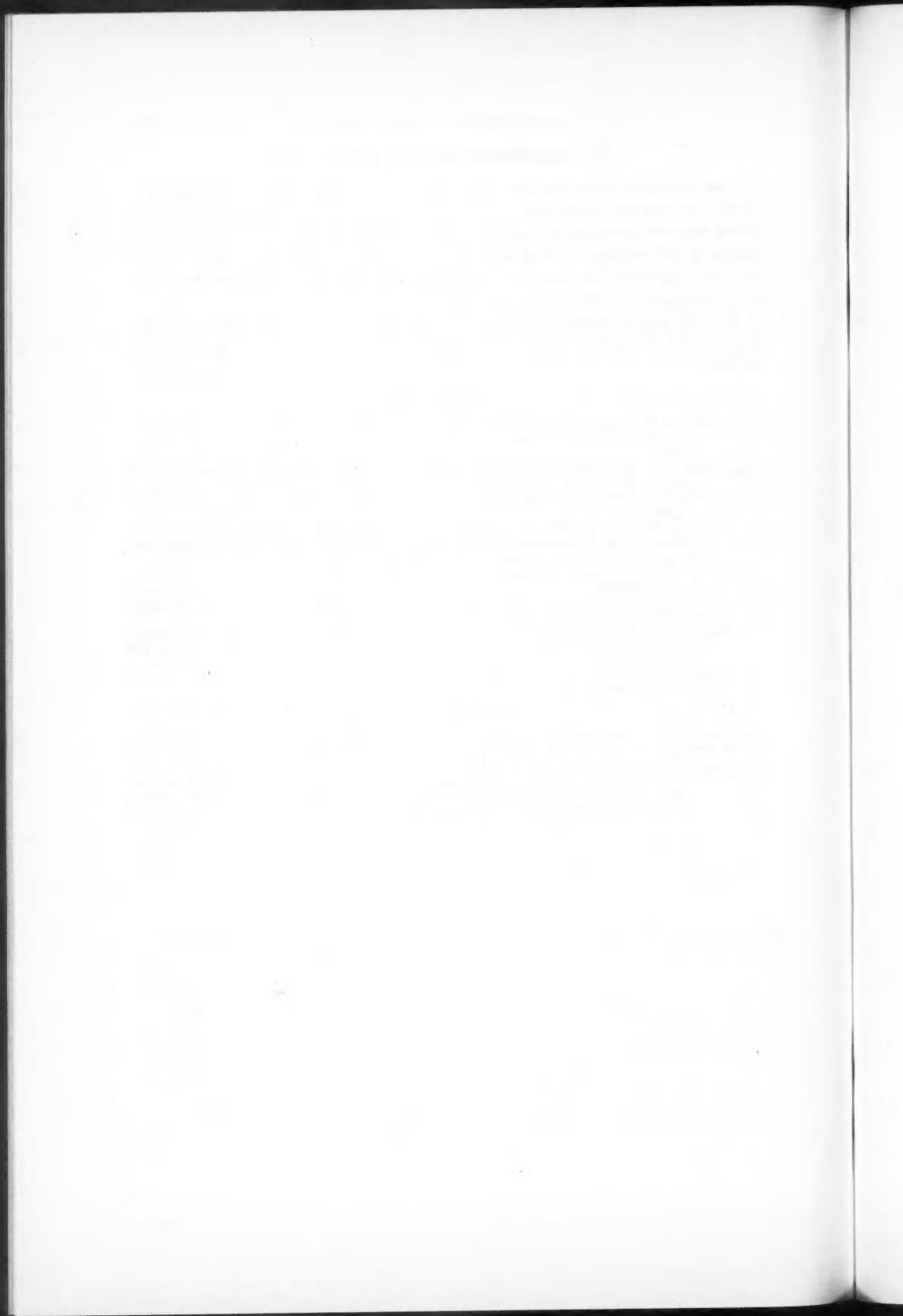
STUDIES ON STRONGYLOIDES OF PRIMATES

IV. EFFECT OF TEMPERATURE ON THE MORPHOLOGY OF THE FREE-LIVING STAGES OF STRONGYLOIDES FÜLLEBORNI[1]

PREMVATI[2]

Abstract

The optimum temperature for the complete development of the free-living and the infective larvae of *Strongyloides fülleborni* is 25° C. Morphological changes are seen at higher or lower temperatures.

The possible morphological changes due to external environmental factors to which the free-living stages of the genus *Strongyloides* are subjected have not yet been reported. Rhabditiform larvae (of either the parasitic or the free-living female) are in a constant process of growth and molt, which precludes a study of the constant effect of temperature on them. It is only when these larvae attain their maximum size (as either free-living sexually mature adults or infective larvae) that they present constant morphological features. It is at this stage, therefore, that morphological changes due to temperature become important.

The optimum temperature for development is 25° C. Complete development of *Strongyloides fülleborni* does not take place at temperatures below 15° C. or above 37° C.

Free-living adults were obtained, by baermanning, from cultures maintained for:

> 96 to 144 hours at 15° C.
> 72 to 96 hours at 20° C.
> 44 to 52 hours at 25° C.
> 44 to 52 hours at 30° C.
> 35 to 40 hours at 35° C.
> 35 to 40 hours at 37° C.

In order to study their comparative morphology, about 100 females were removed from each culture maintained at each of the above-mentioned temperatures and their individual morphological characteristics examined microscopically. As the free-living female is used to differentiate the three species from primates (*S. fülleborni*, *S. cebus*, and *S. simiae*) only the free-living female is considered in detail in this study.

Size of Female

The length varies slightly at different temperatures, ranging from 0.90 to 1.19 mm., the maximum length being attained at between 20 and 30° C. At 15° C. most females have a maximum length of 1.0 mm., and between 35 and 37° C. up to 1.05 mm. Temperature has more effect on width than on length.

Maximum width is attained at 25° C., minimum at 15° C. (Table I).   At 25° C. the maximum width is found just above the vulva, just below which there is a marked constriction giving a waist-like appearance.
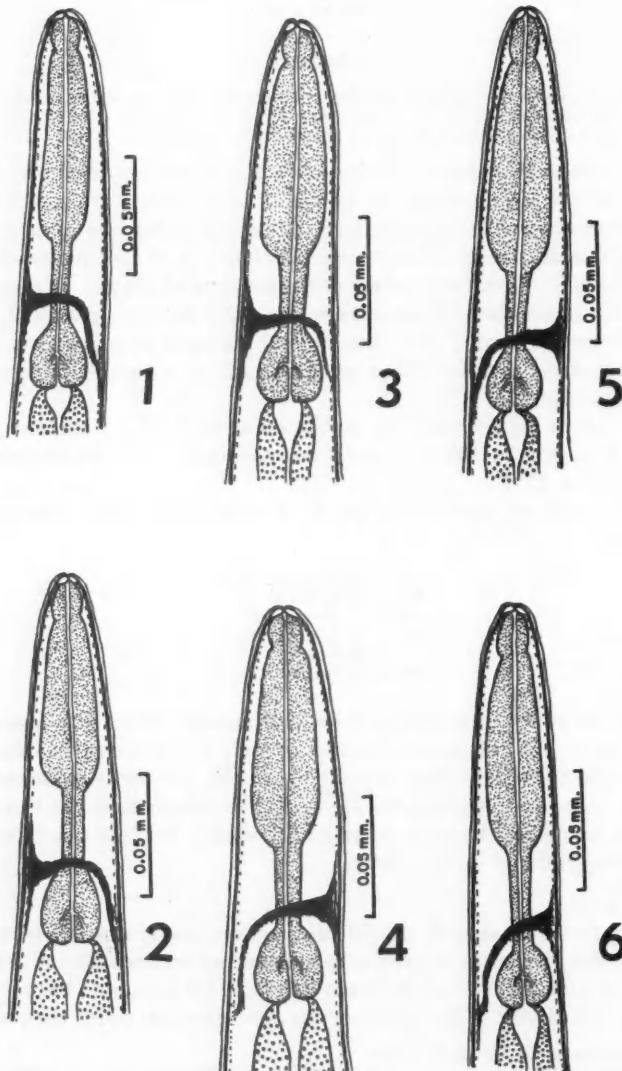
FIGS. 1–6.   Oesophagus of the free-living adult female of *S. fülleborni* developed in cultures maintained at different temperatures.   Fig. 1. At 15° C.   Fig. 2. At 20° C. Fig. 3. At 25° C.   Fig. 4.   At 30° C.   Fig. 5. At 35° C.   Fig. 6. At 37° C.

Oesophagus (Figs. 1–6)

The morphology of the oesophagus varies at different temperatures. While the width of the female is greatest at 25° C. the length of the oesophagus is greatest at unfavorable temperatures. At 25° C. it measures from 0.143 to 0.156 mm., at 37° C. from 0.158 to 0.163 mm. The width of the oesophagus is greatest at 25° C., least at 37° C. In other words, females developed at the optimum temperature have the greatest body length and a shorter broader oesophagus than those developed at suboptimum temperatures. Moreover, at suboptimum temperatures the neck joining the corpus and end bulb becomes narrower and longer. At 25° C. the neck measures 0.029 mm., at 30° C. 0.030 mm., at 35° C. 0.032 mm., at 20° C. 0.035 mm., at 15° C. 0.039 mm., and at 37° C. 0.04 mm. Although there are slight variations in these measurements they are a constant feature in adult females developing at different temperatures.

TABLE I

AVERAGE MEASUREMENT OF 20 FREE-LIVING FEMALES AT
DIFFERENT TEMPERATURES

Temperature	Total length	Width just anterior to vulva	Width just posterior to vulva	Length to oesophagus
15° C.	0.89 to 1.01	0.044 to 0.049	0.044 to 0.046	0.153 to 0.161
20° C.	0.92 to 1.08	0.055 to 0.065	0.046 to 0.055	0.140 to 0.160
25° C.	0.94 to 1.19	0.059 to 0.076	0.046 to 0.052	0.143 to 0.156
30° C.	0.93 to 1.189	0.059 to 0.067	0.052 to 0.057	0.143 to 0.158
35° C.	0.91 to 1.05	0.057 to 0.062	0.052 to 0.057	0.143 to 0.163
37° C.	0.89 to 1.02	0.052 to 0.054	0.049 to 0.052	0.158 to 0.163

NOTE: All measurements are in millimeters.

Vulva (Figs. 7–12)

The most remarkable morphological change in females developing at different temperatures is expressed in the vulva. At 25° C. (Fig. 7) the vulva is characteristic of *S. fülleborni* with a prominent anterior lip and a marked constriction just posterior. The average maximum width attained by the female just above the vulva is 0.059 to 0.076 mm. as compared with 0.046 to 0.052 mm. just below, a very noticeable difference of 0.013 to 0.024 mm. This width gradually decreases with higher and lower temperatures (Table I). At 37° C. the difference is only 0.002 to 0.004 mm.

The shape of the vulva also shows considerable morphological difference. In females developed at 25° C. the vulvar lips are very prominent with the anterior lip protruding over the posterior one, giving it a marked constriction (Fig. 7). At 30° C. the difference in the width at the vulvar region is practically the same but the lips of the vulva and the constriction below the vulva are less marked (Fig. 8). At 35° C. the constriction is further reduced and the waist-like appearance practically missing (Fig. 9): the vulvar lips
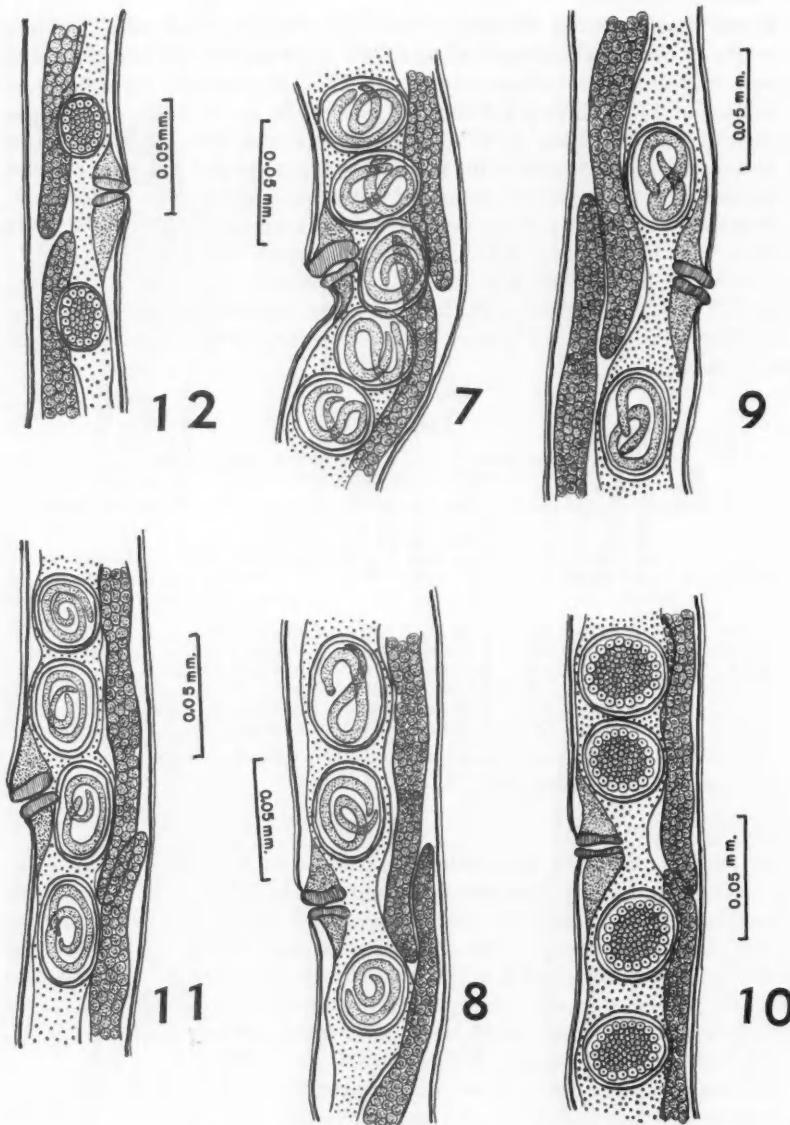
FIGS. 7–12.   Vulvular region of the free-living adult female of *S. fülleborni* developed in cultures maintained at different temperatures.   Fig. 7. At 25° C.   Fig. 8. At 30° C. Fig. 9. At 35° C.   Fig. 10. At 37° C.   Fig. 11. At 20° C.   Fig. 12. At 15° C.

become thinner and of equal size.   At 37° C. the width of the female is almost
the same throughout its length, showing no curve in the vulvar region (Fig. 10).
At 20° C. (Fig. 11) the shape of the vulva is similar to that at 30° C. although
the width is slightly reduced.   At 15° C. the vulva has very salient lips and
there is practically no curve below it (Fig. 12).

Eggs

   The number of eggs in the uterus of the adult female varies with the tem-
perature.   The maximum number (35) in the uterus at one time is reached at
25° C.   The maximum number at 15° C. and 37° C. is never over 10.   The
stage of development of eggs in the uterus also varies with the temperature.
After 44 hours' incubation at 25° C. the eggs are always fully embryonated
and hatch quickly when laid.   No embryonation of eggs in the uterus is seen
at 37° C.; although the eggs reach the maximum size, they do not hatch.
It is possible that the eggs are unfertilized and that this temperature inhibits
their development.   At 15° C. the eggs are generally smaller, the develop-
ment being retarded, and the infective larvae develop from them in from
5 to 7 days.

Infective Larvae

   The only effect of temperature seems to be on size.   The maximum length
of 0.75 mm. is attained at from 25 to 30° C., the minimum of 0.47 mm. at 15° C.
At 25° C. the average length is 0.615 to 0.75 mm. and at 15° C. 0.47 to 0.57 mm.
At 37° C. the eggs of the free-living female do not hatch and there is no indirect
life cycle in the formation of infective larvae.   At this temperature, however,
larvae sometimes show a direct development and the eggs of the parasitic
female develop directly to infective larvae which have an average length of
from 0.52 to 0.58 mm.

   There is considerable variation in the size of the oesophagus in relation to
body length even at the same temperature (0.182 to 0.28 mm.) so the effect
of different temperatures on the relative sizes of oesophagus is not worth
considering.

Discussion

   The effect of temperature on the morphology of the free-living adult female
leads to a suspicion of the validity of the three species (*S. fülleborni*, *S. cebus*,
and *S. simiae*) from primates.   There is no possibility of the monkeys from
which the author's specimens were obtained being infected with different
species of *Strongyloides*: the cultures were all made at one time from the
contents of the intestines of monkeys, and then maintained at different
temperatures.   Moreover, the free-living females developed at the same tem-
perature and the type of structures remained the same.

   The free-living females developed at 25 to 30° C. show all the features
characteristic of *S. fülleborni*: a prominent vulva and a marked waist-like
constriction below the vulva.   Those developed at 20 and 35° C. resemble the
free-living females described for *S. cebus* Darling (1911): a less prominent vulva
and no waist-like appearance.   Those developed at 15° C. resemble perfectly

the descriptions of *S. simiae* Hung and Hoeppli (1923): salient lips and uniform body width in the vulva region. An examination of these free-living females developed at different temperatures would lead an observer to consider them as belonging to different species.

The possible explanation of the morphological changes observed in the free-living females at different temperatures perhaps lies in normal development at some temperatures and modifications in structures at others. The temperature range favoring normal growth is very limited. In nature, the temperature range will vary according to the climate and within certain limits the free-living larvae adapt themselves to adverse conditions.

During the present investigation it was observed that the shape of the vulva depends upon the presence or absence of embryonated eggs, and this in turn depends upon the opportunities for the female to mate a number of times. At above and below 25° C. the motility of the larvae is reduced; there is less possibility of the adults copulating frequently, and the vulva remains less prominent. When the females are separated from the males before mating only non-embryonated eggs are observed in the uteri and these do not hatch. When females and males are separated after the first mating, the first batch of embryonated eggs are laid and a few more in the second batch, but later on the eggs in the uterus do not develop beyond the morula stage. At 15° C. copulation is rare and the shape of the vulva is of the salient type. This finding is confirmed by the fact that the maximum number of infective larvae is obtained at 25° C. from the same number of females and males.

In reviewing the morphological changes observed at different temperatures the author is inclined to believe in the validity of very few species of the genus *Strongyloides*. The slight differences in size and morphology are probably due to the repeated long range of alternative lives under similar conditions of development. The life cycle of *Strongyloides* is a short one: once in the host the infective larvae become fully formed parthenogenetic females in 7 to 10 days and soon begin laying viable eggs. When passed out of the host these eggs are fully embryonated; they hatch and develop to free-living adults in 48 hours. The eggs of the free-living generation develop into infective larvae in 2 days. Thus, the life cycle is completed within 12 to 15 days. Accordingly, if the same adverse environment is met with by succeeding generations the morphological characteristics are so adapted as to enable the parasite to survive in that environment.

Summary and Conclusions

The size of the free-living female depends slightly upon the temperature at which it is cultured. Maximum size is attained at 25° C. The oesophagus in the female developed at this temperature is relatively shorter and broader. At 25° C. the vulva of the free-living female is characteristic of *S. fülleborni*, whereas at 20 and 35° C. it resembles that of *S. cebus*, and at 15° C. *S. simiae*. The number of eggs in the uterus of the free-living adult female differs with the temperature. The maximum number of 35 eggs is observed at 25° C. The size of the infective larva is also dependent upon temperature, the maximum size being attained at between 25 and 30° C.

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   Drawings should be carefully made with India ink on white drawing paper, blue tracing linen, or co-ordinate paper ruled in blue only; any co-ordinate lines that are to appear in the reproduction should be ruled in black ink. Paper ruled in green, yellow, or red should not be used. All lines should be of sufficient thickness to reproduce well. Decimal points, periods, and stippled dots should be solid black circles large enough to be reduced if necessary. Letters and numerals should be neatly made, preferably with a stencil (**do NOT use typewriting**), and be of such size that the smallest lettering will be not less than 1 mm. high when reproduced in a cut 3 in. wide.

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Contents